A STUDY OF PREVALENCE OF DERMATOPHYTES IN NORTH CHENNAI AND A PROFILE OF THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN

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DECLARATION

I, **Dr.M.Suganthi,** solemnly declare that this dissertation "**A STUDY OF PREVALENCE OF DERMATOPHYTES IN NORTH CHENNAI AND A PROFILE OF THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN"** is the bonafide work done by me at the Department of Microbiology, Government Stanley Medical College and Hospital, Chennai, under the guidance and supervision of **Prof.Dr.P.R.THENMOZHI VALLI, M.D.,** Professor of Microbiology, Government Stanley Medical College, Chennai-600 001.

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Place: Chennai. **Dr.M.SUGANTHI**
Date:

INTRODUCTION

The cutaneous infections of man includes a wide variety of diseases in which the integuments and its appendages the hair and the nail are involved. Infection is generally restricted to the non lining cornified layer but a variety of changes occur in the host because of the presence of the infectious agent and its metabolic products. Majority of the infections are caused by a homogenous group of keratophilic fungus called the dermatophytes.(62) A single species might be involved in several clinical types each with its distinct pathology. The fungi are the commonest infective agent of man and no group of people or geographical areas are without taenia or ringworm infection (taenia-latin for worm). Evolutionary development towards an accommodating host parasite relationship can be seen among the dermatophytes which is absent among other fungal agent of human disease. This group of disease is collectively referred to as dermatophytosis.

Dermatophytes are a group of closely related group of organisms that can use keratin as a nitrogen source.(70). On the basis of clinical, morphologic and microscopic charecterteristics three genera are recognized as Dermatophytes; Trichophyton, Microsporum and Epidermophyton. Depending on their natural habitat dermatophytes may be Anthropophilic (people loving), Zoophilic(animal loving) and Geophilic (soil loving).

Dermatophytes includes several distinct clinical varieties, depending on the anatomical site and the etiological agent involved. The pathology induced on the host initially is an eczemiform response followed by allergy and inflammatory manifestations. The type and severity of these reactions are related to the immune status of the host as well as to the strain and species of the organism causing the infection.

Dermatophytosis remains a significant public health problem. Numerous anti fungal agents have been developed since Grisofluvin became available through a breakthrough experimental work of Gentles in guinea pig in 1958. This discovery revolutionized the therapeutic approach to this disease. In 1980 discovery of azoles' and derivatives and allied groups of antifungal drugs had significant impact in the management of dermatophytosis the increasing number of immunosupressed patients and expanding drug resistance of microorganisms make the development and appropriate use of the most important areas in microbiology and infective medicine. The introduction of modern patient management technologies and new and more chemotherapeutic agents and the more aggressive use of chemotherapy have resulted in rapidly expanding patients with chemically induced immunosuppression. These patients became highly susceptible to severe fungal infections. As fungal infections became an important public health problem the resistance to established anti fungal agents also began to emerge. Newer agents of either a broader spectrum or different targets of activity are being developed. The prognosis among these patients is very poor and therefore institution of early diagnosis and treatment is essential.

Now effective drugs are available for chemotherapy of fungal infections and further modalities are being developed for management of the same. Standardization of in vitro susceptibility testing provides a consistent and reproducible data that may predict clinical response when used in conjunction with individual patient risk factors. An ideal antifungal drug should have a broad spectrum activity, it should be effective in vivo and there should be no drug resistance.

The development of national committee for clinical laboratory standards (NCCLS) reference method M38A in the 1990”s has improved the reproducibility of invitro anti fungal susceptibility test data and facilitated the establishment of interpretive break points for the triazoles, fluconazole and itraconazole.

AIMS AND OBJECTIVES

1. To isolate the dermatophytes from clinical specimens like skin, nail and hair obtained from patients attending Dermatology OP.

2. To speciate the isolates of dematophytes.

3. To study the correlation of fungal isolates and the clinical manifestations.

4. To determine the commonest prevalent genus and species of dermatophytes in North Chennai.

5. To determine the antifungal susceptibility of the isolates by different methods

6. To compare the results obtained by different methods

7. To determine the MIC values of different drugs for the isolates

REVIEW OF LITERATURE

History

Historically **Agostino Bassi**(1835-36) was the first to elucidate the microbial nature of decaying disease of silkworms. **Robert Remak** (1873) first observed hypae in the scalp scrapping. **Professor Johann L Schonlein** described the fungal etiology in 1839 and Robert Remak described the etiological agent and named it as Achovion Scholeinleini(Topley). He credited this discovery to his mentor Professor Johann L Scholein. Real founder of Medical Mycology branch based on his discoveries from (1841 - 1844) was the Parisian physician **David Gruby**. He described clinical entities (i.e.) favo, scalp diseases caused by dermatophytes and demonstrated that fungi could be cultured and transmitted to human also.(64)

Domenico Majocehi (1849 -1929) first described variants of Tinea Corporis popularly called as Majorcehi's granuloma which is an uncommon infection of dermal and subcutaneous tissue caused by dermatophytes and he named this disorder as Granuloma Tricofitico in 1883.

In 1892 **Raymond Sabouraud** a French dermatologist established the plurality of ringworm fungi and integrated the mycological and clinical aspects of ringworm. Based on the advances made by his contemporaries in medicine and veterinary science as well as on his personal observations Sabourand wrote and published his monumental Les-Feignus in1910. He

classified dermatophytes into four genus Achovion, Epidermophyton, Microsporum and Tricophyton based on the clinical aspects of the diseases that they caused, combined with their culture and microscopic characters.(78)

In 1925 **Robert W.Wood**, a Baltimore physician invented the Wood's lamp for detection of fungal infection of hair.

In 1934 **Chester Emmons** modified the taxonomic scheme of Sabourand. He discarded the genus Achovion and redefined the three anamorphic genus Epidermophyton, Microsporum and Tricophyton based on the colonial morphology and other relatively reliably formed microscopic structures.

Dawsan and Gentles in1959 using hair bait technique of Vanbreusegham led to discovery of Telomorphs of many dermatophytes and related keratophylic fungi.

In 1958 Gentles discovered Grisofluvin after his work on guinea pigs.

In 1980 discovery of azole derivatives and allied group of antifungal drugs had significant impact on the management of dermatophytons.(32)

Dermatophytes are a group of closely related filamentous fungi and are classified as,

1. Trichophyton

2. Microsporum

3. Epidermophyton

Depending on their natural habitat dermatophytes may be Anthropophilic- Evolved from zoophilic species .Human are the general host and transmission may occur by direct contact or indirectly by fomites (eg) E.flocossum. (77) human (main host)& mild chronic lesions Zoophilic- These dermatophytes have evolved from soil to parasitize animals. (64) Infection in man is accidental through direct or indirect contact. Causes severe inflammation but readily curable(eg)T.verrucosum, T.equinum)

Geophilic- Considered ancestral to the the pathogenic dermatophytes.(68) Natural habitat is the soil, where they are associated with decaying keratinacious material(eg) hair, nail, hooves, feather etc. Exposure to soil is the main source of infection for man and animals.(13). They are less pathogenic(eg) M.gypseum, M.nannum.

The genus Trichophyton, Microsporum and Epidermophyton are the principle etiological agents of Dermatomycosis. Their morphplogy and cultural characters are as follows

Trichophyton sp.

The genus *Trichophyton* is characterized by the development of both smooth-walled macro- and microconidia. Macroconidia are mostly borne

laterally directly on the hyphae or on short pedicels, and are thin- or thick-walled, clavate to fusiform, and range from 4 to 8 by 8 to 50 um in size. Macroconidia are few or absent in many species. Microconidia are spherical, pyriform to clavate or of irregular shape and range from 2 to 3 by 2 to 4 um in size. The different species are Trichophyton ajelloiTrichophyton concentricum Trichophyton equinum, Trichophyton mentagrophyes var. nodulare,Trichophyton mentagrophytes var. erinacei, Trichophyton mentagrophytes var. interdigitale, Trichophyton mentagrophytes var. mentagrophytes, Trichophyton mentagrophytes var. quinckeanum, Trichophyton rubrum, Trichophyton rubrum downy strain, Trichophyton rubrum granular strain,Trichophyton schoenleinii, Trichophyton soudanense(30) Trichophyton terrestre, Trichophyton tonsurans, Trichophyton verrucosum, Trichophyton violaceum

Trichophyton mentagrophytes

On Sabouraud's dextrose agar, colonies are generally flat, white to cream in colour, with a powdery to granular surface. Some cultures show central folding or develop raised central tufts or pleomorphic suede-like to downy areas. Reverse pigmentation is usually a yellow-brown to reddish-brown colour. Numerous single-celled microconidia are formed, often in dense clusters. Microconidia are hyaline, smooth-walled, and are predominantly spherical to subspherical in shape, however occasional clavate to pyriform forms may occur. Varying numbers of spherical chlamydoconidia, spiral hyphae and smooth, thin-walled, clavate shaped,

multicelled macroconidia may also be present.Hair Perforation Test**:** Positive within 14 days(66)

Clinical significance:

T. mentagrophytes var. mentagrophytes is the zoophilic form of T. mentagrophytes with a world-wide distribution and a wide range of animal hosts including mice, guinea-pigs, kangaroos, cats, horses, sheep and rabbits. Produces inflammatory skin or scalp lesions in humans, particularly in rural workers. Kerion of the scalp and beard may occur. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light.

Trichophyton rubrum

Two types may be distinguished: *T. rubrum* downy type and *T. rubrum* granular type. Microscopically, the downy type is characterized by the production of scanty moderate numbers of slender clavate microconidia and no macroconidia. Microscopically, the granular type is characterized by the production of moderate to abundant numbers of clavate to pyriform microconidia and moderate to abundant numbers of thin-walled, cigar-shaped macroconidia. The macroconidia may or may not have terminal appendages.

Clinical significance:

Trichophyton rubrum is an anthropophilic dermatophyte. The downy strain has become the most widely distributed dermatophyte of man. It

frequently causes chronic infections of skin, nails and rarely scalp. The granular strain is a frequent cause of tinea corporis .

Trichophyton schoenleinii

On Sabouraud's dextrose agar, colonies are slow growing, waxy or suede-like with a deeply folded honey-comb-like thallus and some sub-surface growth. The thallus is cream coloured to yellow to orange brown. Cultures are difficult to maintain in their typical convoluted form, and rapidly become flat and downy. No reverse pigmentation is present. No macroconidia and microconidia are seen in routine cultures, however numerous chlamydoconidia may be present in older cultures. However, characteristic antler "nail head" hyphae also known as "favic chandeliers" may be observed. A few distorted clavate microconidia may be formed by some isolates when grown on polished rice grains(78).

Clinical significance: *Trichophyton schoenleinii* is an anthropophilic fungus causing favus in humans. Favus is a chronic, scarring form of tinea capitis characterized by saucer-shaped crusted lesions or scutula and permanent hair loss. Invaded hairs remain intact and fluoresce a pale greenish yellow under Wood's ultra-violet light. Favus is common in Eurasia and Africa.

Trichophyton tonsurans

In Sabouraud's dextrose agar, colonies show considerable variation in texture and colour. They may be suede-like to powdery, flat with a raised centre or folded, often with radial grooves. The colour may vary from pale-buff to yellow, the so called sulfureum form which resembles

Epidermophyton floccosum, to dark-brown. The reverse colour varies from yellow-brown to reddish-brown to deep mahogany. Hyphae are relatively broad, irregular, much branched with numerous septa. Numerous characteristic microconidia varying in size and shape from long clavate to broad pyriform, are borne at right angles to the hyphae, which often remain unstained by lactophenol cotton blue. Very occasional smooth, thin-walled, irregular, clavate macroconidia may be present on some cultures. Numerous swollen giant forms of microconidia and chlamydoconidia are produced in older cultures.(70)Hydrolysis of Urea: positive at 5 days.Hair Perforation Test **:** positive within 14 days.

Clinical significance:

Trichophyton tonsurans is an anthropophilic fungus with a world wide distribution which causes inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails and scalp. Invaded hairs show an endothrix infection and do not fluoresce under Wood's ultra-violet light.

Trichophyton verrucosum

On Sabouraud's dextrose agar, colonies are slow growing, small, button-or-disk-shaped, white to cream coloured, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth. Reverse pigment may vary from non-pigmented to yellow. Broad, irregular hyphae with many terminal and intercalary chlamydospores. Chlamydospores are often in chains. The tips of some hyphae are broad and

club-shaped, and occasionally divided, giving the so-called "antler" effect. When grown on thiamine-enriched media, occasional strains produce clavate to pyriform microconidia borne singly along the hyphae. Macroconidia are only rarely produced, but when present have a characteristic tail or string bean shape.

Growth at 37C**:** unlike other dermatophytes growth is enhanced at 37C

Clinical significance:

Trichophyton verrucosum is a zoophilic fungus causing ringworm in cattle. Infections in humans result from direct contact with cattle or infected fomites and are usually highly inflammatory involving the scalp, beard or exposed areas of the body (ie. nails, skin). Invaded hairs show an ectothrix infection and fluorescence under Wood' ultra-violet light has been noted in cattle but not in humans. Geographic distribution is world-wide.

Trichophyton violaceum

On Sabouraud's dextrose agar, colonies are very slow growing, glabrous or waxy, heaped and folded and a deep violet in colour. Cultures often become pleomorphic, forming white sectors and occasional non-pigmented strains may occur. Hyphae are relatively broad, tortuous, much branched and distorted. Young hyphae usually stain well in lactophenol cotton blue, whereas older hyphae stain poorly and show small central fat globules and granules. No conidia are usually seen, although occasional

pyriform microconidia have been observed on enriched media. Numerous chlamydoconidia are usually present, especially in older cultures.

Clinical significance:

Trichophyton violaceum is an anthropophilic fungus causing inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails, beard and scalp, producing the so-called "black dot" tinea capitis. Invaded hairs show an endothrix infection and do not fluoresce under Wood's ultra-violet light.

Microsporum sp.

Microsporum species form both macro- and microconidia on short conidiophores. Macroconidia are hyaline, multiseptate, variable in form, fusiform, spindle-shaped to obovate, ranging from 7 to 20 by 30 to 160 um in size, with thin- or thick- echinulate to verrucose cell walls. Their shape, size and cell wall features are important characteristics for species identification. Microconidia are hyaline, single-celled, pyriform to clavate, smooth-walled, 2.5 to 3.5 by 4 to 7 um in size and are not diagnostic for any one species.Seventeen species of *Microsporum* have been described, however only the more common species are included in these descriptions.*Microsporum audouinii Microsporum canis, Microsorum canis var. distortum, Microsporum cookei, Microsporum equinum, Microsporum ferrugineum, Microsporum fulvum, Microsporum gallinae, Microsporum gypseum, Microsporum nanum, Microsporum persicolor.,*

Clinical significance:

The genus *Microsporum* contains a number of important species that are the principle causative agents of animal and human dermatophytoses [tinea and ringworm](14).

Microsporum gypseum

On Sabouraud's dextrose agar, colonies are usually flat, spreading, suede-like to granular, with a deep cream to tawny-buff to pale cinnamon coloured red surface. Many cultures develop a central white downy umbo (dome) or a fluffy white tuft of mycelium and some also have a narrow white peripheral boarder. A yellow-brown pigment, often with a central darker brown spot, is usually produced on the reverse, however a reddish-brown reverse pigment may be present in some strains. Cultures produce abundant, symmetrical, ellipsoidal, thin-walled, verrucose, 4-6 celled macroconidia. The terminal or distal ends of most macroconidia are slightly rounded, while the proximal ends (point of attachment to hyphae) are truncate. Numerous clavate shaped microconidia are also present, but these are not diagnostic.

Clinical significance:

Microsporum gypseum is a geophilic fungus with a world-wide distribution which may cause infections in animals and humans, particularly children and rural workers during warm humid weather. Usually produces a

single inflammatory skin or scalp lesion. Invaded hairs show an ectothrix infection but do not fluoresce under Wood's ultra-violet light(1).

Microsporum canis

On Sabouraud's dextrose agar, colonies are flat, spreading, white to cream-coloured, with a dense cottony surface which may show some radial grooves. Colonies usually have a bright golden yellow to brownish yellow reverse pigment, but non-pigmented strains may also occur. Macroconidia are typically spindle-shaped with 5-15 cells, verrucose, thick-walled and often have a terminal knob. A few pyriform to clavate microconidia are also present. Macroconidia and/or microconidia are often not produced on primary isolation media and it is recommended that sub-cultures be made onto boiled polished rice grains to stimulate sporulation.Hair Perforation Test**:** Positive at 14 days.

Clinical significance:

Microsporum canis is a zoophilic dermatophyte of world-wide distribution which is a frequent cause of ringworm in humans, especially children. Invades hair, skin and rarely nails. Cats and dogs are the main sources of infection. Invaded hairs show an ectothrix infection and fluoresce a bright greenish-yellow under Wood's ultra-violet light(15).

Microsporum nanum

Colonies are flat, cream to buff in colour with a suede-like to powdery surface texture. Young colonies have a brownish-orange pigment

which deepens into a dark reddish-brown with age. Cultures produce numerous small ovoid to pyriform macroconidia with 1-3 cells, but mostly 2 cells, with relatively thin, finely echinulate (rough) walls, and broad truncate bases. Many macroconidia are borne on conidiophores (stalks) which do not stain readily. Occasional clavate microconidia are present, which distinguishes *M. nanum* from some species of *Chrysosporium*.

Clinical significance:

Microsporum nanum is a geophilic and zoophilic fungus frequently causing chronic non-inflammatory lesions in pigs and a rare cause of tinea in humans. Also present in soil of pig-yards. Infections in man usually contacted directly from pig or fomites. Invaded hairs may show a sparse ectothrix or endothrix infection but do not fluoresce under Wood's ultra-violet light. The geographical distribution is world-wide.

Epidermophyton floccosum

On Sabouraud's dextrose agar colonies are usually slow growing, greenish-brown or khaki coloured with a suede-like surface, raised and folded in the centre, with a flat periphery and submerged fringe of growth. Older cultures may develop white pleomorphic tufts of mycelium. A deep yellowish-brown reverse pigment is usually present. Microscopic morphology shows characteristic smooth, thin-walled macroconidia which are often produced in clusters growing directly from the hyphae. Numerous chlamydoconidia are formed in older cultures. No microconidia are formed(32).

Clinical significance:

Epidermophyton floccosum is an anthropophilic dermatophyte with a world-wide distribution which often causes tinea pedis, tinea cruris, tinea corporis and onychomycosis. It is not known to invade hair in vivo and no specific growth requirements have been reported. *E. floccosum* infections may become epidemic among personnel using common shower or gym facilities, e.g. athletic teams, troops, ship crews and inmates of institutions.

PATHOGENESIS AND PATHOLOGY

Dermatophytes are not endogenous pathogens. Transmission of dematophytes occur via three sources, each resulting in typical features.

TYPES OF DERMATOPHYTES BASED ON MODE OF TRASMISSION		
Category	Mode of transmission	Typical clinical features
Anthropophilic	Human to human	Mild non inflammatory, chronic
Zoophilic	Animal to human	Intense inflammation (pustules and vesicles possible), acute
Geophilic	Soil to human or animal	Moderate inflammation

Dermatophytes typically invade only the outer cornified layer of the skin they can be responsible for considerable morbidity. Thiers adaptation different host has evolved, greater chronicity and further spread of infection.

Dermatophytes, unlike most other fungi produce keratinase (enzymes that break down keratin) which allow for invasion of fungi to keratinized tissue. Mannans in the cell wall of dermatophytes have immuno-inhibitory effects. In T.rubrum the mannans also decrease proliferation, therefore decreasing the likelyhood of fungus being sloughed off prior to invasion .This is thought to contribute significantly to the chronicity of infection caused by T.rubrum. The invasion of dermatophyte is subject to host factors including protease inhibitors and possibly harmones which may limit the extend of infection. The severity of clinical disease is affected by several host factors

sebum has an inhibitory effect on dematophytes

breaks in skin barrier or macerated skin encourage dermatophyte invasion

susceptibility may be inherited

competency of the immune system.

Once the pathogen has invaded and begun to proliferate there are factors that aid in limiting the infection to the keratinized tissue.these include the preference of dermatophytes to cooler temperature of skin compared to the normal body temperature, the presence of factors in serum that inhibit dermatophyte growth (e.g ß globulins, ferritin other metal chelators) and the host immune response. Fungal metabolic products diffuse through the Malphigian layer to cause erythema, vesicls and even pustule formation along with pruritis. The hyphae become old break into arthrospores which are shed off. This is partially responsible for the central

clearing of the ringworm infection. Their in vivo activity is restricted to the zone of differentiation, newly differentiated keratin and for the infection to persist the hyphal growth must keep in pace with the rate of keratin production. The hyphal tips growing within the shaft reach to the edge of living keratinized cells and form **Adamson's fringe**. The infective process ceases and cure occurs when the balance of host and fungus is disturbed in favour of the host and the upward movement of keratin carries the active hyphae away from the keratinatious zone.(32)

CLINICAL FEATURES

The clinical manifestations of dermatophytosis are called **tinea** or **ring worm** depending on the anatomical site involved. The term tinea derived from the Latin word meaning **'worm'** or **'moth'**. This is used descriptively because of the serpentine (snake like) and circular or annular(ring like) lesions that occur in skin. The literal meaning of the word '**dermatophyte'** is **'skin plant'** which is a misnomer as the fungi are phytogenitically not related to plants.(32)

The dermatophytes have distinct clinical manifestations in different parts of the body. The wide variations of clinical presentation depend upon the species and probably the strain of fungus concerned, size of the inoculum, site of body infected and the immune status of the host. The following common clinical conditions are produced by the dermatophytes;

TINEA CORPORIS

Is the dematophytic infection of the skin of the trunk and extremities excluding the hair, nail, palms , soles and groin. Any dermatophyte can potentially cause Tinea corporis , but T.rubrum is the most common pathogen worldwide followed by T.mentagrophytes. Spread from human to human, animal to human or soil to human. Domestic animals are the important factors in transmission of organisms causing tinea corporis specifically the zoophilic types. Important risk factor in acquiring Tinea corporis is having personal history of or close contact with t\Tinea capitis or Tinea pedis. Other predisposing factors include occupational or recreational exposure (military housing, gymnasiums, locker rooms, outdoor occupations, wrestling),contact with contaminated clothings and furniture and immunosuppression. Incubation period 1 to 3 weeks. Lesions may be arcuate ,circinate oval in shape , scaly(lessened or absent in corticosteroid use –tinea incognito). Lesions may be vesicular, granulomatous or verrucous in appearance. Associated symptoms include pruritis and burning. Clinical variants of tinea corporis include tinea profunda, Majjochi's granuloma and tinea imbricate. Tinea profunda results from excessive inflammatory response to a dermatophyte(analogous to a kerion on the scalp) . Majjoci's granuloma caused by T.rubrum is characterized by perifollicular pustules or granulomas. T.imbricata caused by T.concentricum is a chronic infection presenting as concentric annular rings.

TINEA CRURIS

Is the dermatophyte infection of the inguinal region particularly inner aspect of upper thigh and crural folds. Three most common causative agents are E.floccosum, T.rubrum and T.mentagrophytes. Men more commonly

affected –scrotum encourages a warm and moist environment. Other predisposing factors are obesity and excessive perspiration. Initial sign is an area of erythema and pruritis in the intertrigenous fold between the scrotum and thigh. Followed by sharply demarcated with a raised erythematous scaly advancing border, border may contain pustules or vesicles.

TINEA MANNUM

Is the infection of the palm and the inter digital spaces(thought to be related to the lack of sebaceous glands in the palm. Causative organisms - E.floccosum, T.rubrum and T.mentagrophytes. usually associated with Moccasin type tinea pedis .

TINEA BARBAE

Dermatophytosis limited to postpubertal males and involves the bearded areas of face and neck. Causative organism is T.mentagrophytes var mentagrophytes and T.verrucosum. Others are T.schonleinii, T.violaceum and T,megninii. Common cause of infection was contaminated razors in barber shops. Because zoophilic organisms are the common cause and due to the large amount of terminal hair follicles in the affected areas the clinical presentation tends to be severe with intense inflammation and multiple follicular pustules. Abcesses, sinus tracts, bacterial super infection and even kerion like lesion may develop.

TINEA CAPITIS

Common dermatophyte infection of the scalp in children. Causative pathogens T.tonsurans, M. canis, M. audouinii. Three pattern of invasion exists;

Endothrix pattern results from infection with anthropophilic fungi in the genus Trichophyton and is characterized by nonfluorescent arthroconidia within the hair shaft. The clinical presentation varies from scaling to 'black dots' with patchy alopecia to kerion formation. T.tonsurans and T.violaceum are important causes of endothrix formation.

Ectothrix pattern occurs when arthroconidia are formed from fragmented hyphae outside the hair shaft. cuticular destruction ensues. ectothrix infection may be fluorescent(Microsporum) or nonfluorescent (Microsporum and Trichophyton) as determined by wood's lamp examination.

Favus in most severe form of dermatophyte hair infection. Caused by T.schoenleinii. Hyphae and air spaces are observed within the hair shaft , and a bluish white fluorescence under wood lamp examination Favus presents as thick yellow crusts composed of hyphae and skin debris (scutula). Scarring alopecia may develop in chronic cases.

TINEA PEDIS

Is the dermatophyte infection of the soles and interdigital web spaces. Most common infection around the world affecting both the sexes. the lack of sebaceous glands and the moist environment caused by occlusive shoes are the important factors in development of tinea pedis. Most believe that it is acquired by walking bare foot. No specific susceptibility has been determined to explain why some people are more likely to than others to acquire the disease despite the same level of exsposure.commen pathogens – T.rubrum, T.mentagrophytes, E.floccosum and T.tonsurans.there are four types of tinea pedis

- Moccasin
- Interdigital
- Inflamatory
- Ulcerative

TINEA UNGUIUM (ONYCHOMYCOSIS)

Onychomycosis comprises of all fungal infections affecting the nail apparatus, i,e, nail matrix, nail plate, cuticle, mesen chymal tissue and nail folds(29). In spite of improved personal hygiene and living environment onychomycosis continues to spread and persist. The prevalence rate of onychomycosis is determined by age , predisposing factor, social class, occupation, climate, living environment and frequency of travel.(44) It is

divided into three patterns based on the point of fungal entry into the nail unit

- Distal / lateral subungual with invasion via the hyponychium (most common)
- White superficial with direct invasion into the superficial nail plate (often due to T.mentagrophytes)
- Proximal subungual with direct invasion under the proximal nail fold (immunocompromised host)

The most commen pathogens are T.rubrum, T.mentagrophytes and E .floccosum (less frequently Microsporum spp).A single nail may be involved but more commonly multiple nails on one or both hands or feet affected. The responsible organism initially invades the nail bed in the region of hyponychium leading to hyperkeratosis of the nail bed . With further progression of infection there is yellowing and thickening of the distal nail plate as well as onycholysis which is an ideal environment for further proximal invasion and growth of the dermatophyte. Eventually the entire nail bed and plate may become involved (total dystrophic pattern). Serious complication such as cellulitis may arise from onychomycosis especially in patients who are diabetic or immunocompromised.

The treatment of onychomycosis has been attempted throughout ages but only the last two decades has safe and effective systemic treatments has been available for this chronic superficial fungal disease. Oral griseofulvin

and ketoconazole once agents of choice for treatment of onychomycosis has been superseded by newer systemic compounds that have higher cure and lower relapse rate. (48)

Laboratory Diagnosis

In the laboratory, diagnosis depends on the demonstration of causative pathogen in tissue by microscopy isolation of fungus in culture and the serological tests.

Wood's lamp examination

Wood's lamp is a device that is useful in the diagnosis and management of superficial cutaneous fungal infections.

Fluorescence Seen under Wood's lamp

Micro organisms	Fluorescence Colour
Microsporum canis	Bright green
Microsporum audouinii	Bright green
Microsporum ferrugineum	Blue green
Microsporum distortum	Blue green
Microsporum gypseum	Dull yellow
Trichophyton sehoenleinii	Dull green

Direct examination

The microscopic examination of KOH wet mounts of keratinous material is simple and reliable. The clinical materials like skin scales, nail clippings and hair stubs are usually examined.

Nail clippings stained with periodic acid – Schiff stain or grocott's methenamine silver stains are more rewarding as compared to KOH wet mount.(32)

Fungal culture

The clinical specimen should be inoculated on fungal culture irrespective of the direct examination findings. Dermatophytes can grow easily on sabouriud dextrose agar with antibiotics and cycloheximide and media are incubated at 25 degree C, 30 degree C and 27 degree C. The dermatophytes test medium (DTM) at 25 degree C is used to isolate and distinguish dermatophytes from the fungal or bacterial contaminents found in cutaneous lesions. They turn the medium red by raising the pH through metabolic activity while most fungi and bacteria do not.

Hair Perforation Test is performed to differentiate between T.Mentagrophytes and T.Rubrum as well as M.Canis and M.Equinum, respectively. The test is taken as positive when the dermatophyte species show wedge – shaped perforations in the hair. It is positive in T.Mentarophytes and M.Canis and negative in T.Rubrum and M.Equinum.

Urease Test is done on Christensen's medium. Trichophyton mentagrophytes strain, hydrolyse yrea and the medium becomes deep red while T.Rubrum shows negative result.

Immunodiognosis :-

The skin tests with dermatophytic antigen, trichophytin and serological tests are important for the diagonosis of the dermatophytosis.

Various serological test like immunodiffusionnare done to establish the diagnosis of dermatophytosis.

Molecular analyses

The PCR-fingerprinting for the identification of species and varieties of common dermatophytes and related fungi utilizing as a single primer the simple repetitive oligonucleotide (GACA).

Chitin synthase 1 (CHSI) gene analysis of dermatophytes has also been utilized.

Animal Pathogenicity :-

This is useful for the laboratory study of nature of lesions and immunity produced by the organism.

Animal pathogenicity .Testing is done on the guinea pigs. Microsporum canis, M.Gypseum and T.Mentagrophytes may be established more readily in the laboratory animals as compared to other species. The hairs of the area to be infected (usually the dorsal part) are shaved and the skin is scarified before applying the conidial and hyphal suspensions. The lesions develop within a week and resolve after three to four weeks in most of the cases.

ANTIFUNGAL AGENTS USED IN DERMATOPHYTOSIS.(32)

Antifungal antibiotics

Griseofulvin – It inhibits fungal mitosis by interference with polymerized microtubule and spindle formation in dividing cells. It is a fungi static drug and very effective in all forms of dermatophytosis

Synthetic antifungal agents

Thiocarbomate-Tolnaftate, It can be used simultaneously with systemic therapy in Tinae corporis and Tinea cruris.

Allylamines-. These agents selectively inhibits the key enzyme squalene epoxidase which is required for fungal ergosterol biosynthesis and leads to accumulation of squalene which leads to weakening of cell membrane and cell death. The following allylamines are clinically significant Naftifine ,Naftifine and Terbinafine. Terbinafine has high potency against dermatophytes as shown by its very low MIC values. The

fungicidal property, combined with its ability to penetrate rapidly stratum corneum and the nail plate , means that a short course treatment is feasible with a low potential for relapse.

Benzylamines

Butinafine – is a new benzlamine derivative with clinical structure and mode of action similar to allylamine antifungal agents.

Azoles –The principal mode of action of the azole is the inhibition of cytochrome P-450 dependent C14 demethylation in the synthesis of ergosterol of fungal cell membrane. All the azoles interact with the cytochrome P-450 enzyme system in fungal cells resulting in impaired ergosterol synthesis , accumulation of abnormal sterols, a defective cell wall and ultimate fungal death. The azoles are **Imidazoles , Miconazole Clotrimazole, Ketoconazole and Econazole**

B-**Triazoles-Fluconazole**-has poor water solubility, oral absorbtion,extensive bio-availabilty independent of food or gastric pH least protein binding has sufficiently long half life to allow once a day administration, On preliminary clinical studies have reporded it to be effective against dermatophytes.(24)

Miscellaneous antifungal agents –Cyclopiroxolamine, Undecyclonic acid, Whitfield's ointment.

INVITRO SUSCEPTIBILITY TESTING OF DERMATOPHYTES

The National Committee for Clinical Laboratory Standards (NCCLS) M-38-A which describes the standard parameters for testing MIC of established agents against filamentous fungi .This has been latter modified for testing dermatophytes M-38-P with several important factors , such as temperature (28 versus 35ºC) and time of incubation (4 to 10 days versus 21 to 72 hours) (54)

Antifungal susceptibility testing is receiving increased attention with the advent of newer anti fungal drugs . However susceptility testing of filamentous fungi is not as advanced as susceptibility testing . In vitro susceptibility tests should provide a reliable measure of relative activity of the anti fungal agent , correlate with in vivo activity and predict the likely outcome of the therapy, provide a means with which to monitor the development of resistance and predict the therapeutic potentials of newer drugs(58).

In vitro susceptibility testing of fungi, is influenced by a number of technical variables such as inoculum size and preparation. medium composition and pH. duration and temperature of incubation and MIC end point determination. in addition, there are problems unique to fungi like their slow growth rates and the ability of some of them to grow either as years with blastoconidia or as moulds with a variety of conidia depending on pH, temperature ang medium composition. the trailing end point observed

with the azoles is another major problem encountered in the susceptibility testing of the fungi.

Several studies have attempted to correlate the MIC results with outcome. however, only little evidence is available test results with in vitro outcomes.

The retrospective nature of the studies, the documented variability of the non-standardized in vitro methods and the difficulty in defining mycoses and their responses to therapy are responsible for this status(48).

The methods that have been most frequently applied to antifungal susceptibility testing are –Disc diffusion method , Episilometer test, Agar dilution test ,Micro and macrobroth dilution test , spectrophotometric methods and flowcytometry.

MATERIAL AND METHODS

STUDY DESIGN: Prospective Cohort study

The present study was carried out in department of microbiology in Govt .Stanley Medical College and Hospital , Chennai.

STUDY PERIOD:

Over a period of one year from May 2008 to June 2009

SAMPLE SPECIFICATIONS

Skin scrapings ,hair and nail were collected from one hundred and seventy patients who attended the mycology section in the Dermatology out patient department at Stanley Medical College and hospital Chennai.

INCLUSION CRITERIA

All patients with clinically diagnosed dermatophytosis irrespective of age and sex who are not under going treatment for the same.

EXCLUSION CRITERIA

All patients with ring worm infection who are under going treatment

HISTORY ELLICITED FROM THE PATIENT

All relevant details like age ,sex ,duration of complaint ,distribution of lesion and history of previous similar complaints and treatment history. All details about general health and treatment history for Diabetis Tuberculosis

Neoplasms , HIV, surgeries .etc. Detailed history of exposure to animals , known cases , pets at home or any other suspected sources.

SPECIMEN

The specimens were

- Skin scrapings
- Nail clippings
- Hair

METHODOLOGY

COLLECTION OF SPECIMEN

FROM THE SKIN

The affected area was thoroughly swabbed with 70% alcohol to remove surface contaminants .The alcohol was allowed to dry by evaporation. The active edge of the lesion was scrapped with a flame sterilized blunt scalpel . the blunt scalpel was immersed in spirit and passed over flame till the spirit was burnt off. Scalpel allowed to cool before use. The scrapings were collected from the margins of lesion without injuring the skin . this was accomplished by scrapping with the blade kept flat on the skin and not in an angular fashion.(47)

FROM THE SCALP

The same procedure was followed as for skin scrapings; in addition, a few affected hair were also epilated and collected with a pair of flame

sterilized tweezers. Care was taken to collect the basal portion of the hair as fungus was usually found in this area.

FROM THE NAIL

Nail clippings taken from the discolored, dystrophic or brittle parts of the nails. The affected nail was meticulously swabbed with 70% alcohol. After which the nail was clipped or scrapped deeply enough to obtain recently invaded nail tissue.(12)

TRANSPORT OF SAMPLES

The samples were collected in dark paper sachets for transport to laboratory so that the scales may be seen easily. These papers were sterilized in autoclave for 15 minutes at 121˚C.

PROCESSING OF SPECIMEN

DIRECT EXAMINATION

KOH Wet mount – a small amount of specimen was placed on a glass slide with 1-2 drops of10-20% KOH solution. A cover slip was placed over the mixture .This cleared the material within 5-20 minutes, depending on its thickness , warming over a low flame hastens the digestion of keratin. A drop of LPCB (Lacto Phenol Cotton Blue) was added for better visualization of hyphae. The wet mount was examined under low power and high power objectives of light microscope for presence of hyphal elements. The ring worm fungi was differentiated from epidermal cell outlines ,elastic ,cotton and vegetable fibres and artifacts such as intra cellular cholesterol.

All the samples collected were inoculated on to Sabourauds Dextose agar(Emmon “s modification) containing chloramphenicol (50mgs /lt) and cyclohexamide (500 mgs/lt) and into a second tube of SDA with gentamycin to detect the growth of other non dermatophytes in the clinical sample(j.for gent). The slopes were incubated at 25˚C and examined at intervals for evidence of fungal growth . Slopes not showing growth for 4 weeks were discarded. The isolates were inoculated on to Potatoe dextrose agar for better connidiation , the isolates were also inoculated on to Blood agar slopes and modified Christensen’s medium for differentiation of species

FUNGAL SLIDE CULTURE

The slide culture was performed using islotes . The slide culture is used to study undisturbed morphological details particularly relationship between reproductive structures like conidia , conidiophore and hyphae. Fungal slide culture was performed in cases with doubtful morphology.(bailey &scott)

Placed a sterile microscope slide on a bent glass rod at the bottom of a petri dish. A piece of one square centimeter block of Sabouraud dextrose agar or potato dextrose agar was put up on the slide. Inoculated the fungal strain under identification at four sides of agar block. Covered the inoculated block with sterile coverslip and incubatd at 25˚C in BOD incubator. Added a little water on the filter paper to avoid drying of agar.

When growth appears, placed a drop of LCB on a slide and coverslip from block. Likewise a drop of stain was placed on a glass slide of this slide

culture after removing the agar block and placed a fresh coverslip and examined the details microscopically to identify the fungus. The mycelia which adhere to the glass surface usually show characteristic microscopic appearance which may be lost if teasing needle are used as happens in the routine LCB mounts. The slide culture was also directly examined by putting under low power of microscope.

The cellophane tape preparation has come into greater use to overcome the obstacles of time consumption and requirement of the extra equipment to prepare the slide culture. A piece of tape is gently laid over a portion of the fungal colony and slowly lifted to remove an area of the LCB on a microscopic slide and covered with coverslip. This preparation becomes an instant slide culture , revealing relationship of the various fungal structures

HAIR PERFORATION TEST:

This test is done to differentiate between T.mentagrophtyes and T.rubrum as well as M.canis and M.equinum. It is positive in T.mentagrophytes and M.canis and negative in T.rubrum and M.equinum. Placed a filter paper strip into bottom of a sterile distilled water. Added a small portion of sterilized prepubertal or infant hair into water. Added 5-6 drops of 1% yeast extract to speed up reaction. Inoculated colony directly on hair and incubated at 25˚C for four weeks. Observed hair by making a wet smear for prescence of conical perforations of hair shaft.(32)

UREASE TEST ;

This test is done on Christensens medium.This is done to diffentiateT.mentagrophytes from T.rubrum . T,mentagrophytes stran , hydrolyse urea and the medium becomes deep red while T.rubrum does not hydrolyse urea. Urea broth may also be used which is more sensitive.

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR

DERMATOPHYTES

AGAR DILUTION METHOD

REQUIREMENTS:

Sterile test tubes for drug dilution / inoculum preparation /agar slopes with drug dilution/ Micro pipette / sterile tips/ Gloves / disposable face masks

MEDIUM:

Nutrient agar

ANTIFUNGAL DRUGS

Fluconazole (64 – 0.0625 µl), Ketaconazole (32 – 0.0313 µl), Itraconazole (32 – 0.0313 µl), Terbinafine (32 – 0.0313 µl) and Griseofulvin (32 – 0.0313 µl) (18,19,20)

INOCULUM PREPARATION

7-15 days old cultures grown on SDA at 25° C was taken. Mature colonies were covered with 10ml of sterile saline (0.85%.) Growth scraped by sterile Pasteur pipette. Heavy particles were allowed to settle for 15-20 minutes at room temperature . Supernatant mixed with a vortex for 15 seconds. Turbidity of supernatant was adjusted spectrophotometrically to 530 nm 65 -70% absorbance .(76)

ANTI FUNGAL STOCK SOLUTION

Stock solutions of each drug were prepared at a initial concentration of 1000µg/ml.Water insoluble drugs like Griseofulvin, ketoconazole, itraconazole & terbinifine dissolved in DMSO. Fluconazole dissolved in sterile distilled water.Further dilutions to get the required dilutions for each drug is made in distilled water.(54)

TEST PROCEDURE

1.8ml of molten nutrient agar poured into sterile test tubes. Allowed to cool to 50°C.0.2ml of drug dilutions from stock solutions added in descending concentration to NA slopes. 10µl of standardized inoculum added to all tubes except sterility control tube. Tubes incubated at 35°C for 7 days Visualized macrosopically for growth.Lowest concentration of the drug which permitted no macroscopically visible growth after 7 days is taken as MIC.

MICROBROTH DILUTION METHOD

REQUIREMENTS:

Sterile test tubes for drug dilution / inoculum preparation , Sterile disposable microtitre plates, Micro pipette / sterile tips /Gloves / disposable face masks

MEDIUM

RPMI 1640 with glutamine, without bicarbonate in MOPS (3N-Morpholino propane sulphonic acid), buffer sterilized by membrane filteration.(studies)

ANTI FUNGAL STOCK SOLUTION

5ml stock solution prepared for each drug.

For water soluble drugs (eg Fluconazole)

Twofold dilutions of a water soluble antifungal agent is used , they may be prepared volumetrically in broth (table 2-appendix)

For water insoluble drugs-diluent DMSO

For example ,to prepare for a broth microdilution test series containing a water-insoluble drug that can be dissolved in DMSO, for which the highest desired test concentration is 16µg/ml , first weigh 4.8 mg (assuming 100% potency) of antifungal powder and dissolve in 3.0 ml of DMSO. This will provide a stock solution at 1,600µg/ml.Next prepare further dilutions of this

stock solution in DMSO (see table 1).The solutions in DMSO will be further diluted 1:50 in the test medium and a further two fold when inoculated. (54)

DRUG DILUTION

To prepare 5ml volumes of antifungal agent first pipette 4.9 ml volumes of RPMI 1640 medium into each of 10 sterile test tubes. Now, using a single pipette add 0.1 ml of DMSO alone to one 4.9 ml lot of medium (control medium), then 0.1 ml of lowest (3.13microgram/ml) drug concentration in DMSO, then 0.1 ml of the 6.25 µg /ml concentration and continue in sequence up the concentration series, each time adding 0.1 ml volumes to 4.9 ml medium. These volumes were adjusted according to the total No. of test required. Because there will be 1:2 dilution of the drug when combined with the inoculum, the working antifungal solutions are 2 fold more concentrated than the final concentration(NCCLS)

INOCULUM PREPARATION

7-15 days old cultures grown on SDA at 25° C was used. Mature colonies were covered with 10ml of sterile saline (0.85 %). Growth scraped by sterile Pasteur pipette. Heavy particles allowed to settle for 15-20 minutes at room temperature . Supernatant was mixed with a vortex for 15 seconds. Turbidity of supernatant was adjusted spectrophotometrically to 530nm 65 -70% absorbance. Each suspension was diluted 1: 50 in RPMI 1640 .(studies)

INOCULATING RPMI -1640 MEDIUM

Each well will be inoculated on the day of test with 0.1 ml of 2x inoculum suspension. This step will dilute the drug concentration, inoculum densities, and solvent used to the final desired test concentration.

The growth control wells contains 0.1ml of the corresponding diluted inoculum suspension and 0.1ml of the drug diluent without anti fungal agents

TEST PROCEDURE

Test was performed in sterile microtitre plates. Aliquots of 100µls of drug dilutions inoculated in 1-10 microtitre wells. Concentration of Fluconazole 0.01 -64 µg/ml (Concentration of others 0.0039-16µg/ml). Added 100 µl of inoculum into each well from 1 to 12.Growth control -tube 12 with inoculum and without antifungal drug.(25,34,55,)

INCUBATION

All microdilution trays were incubated at 28ºC without agitation.

READING RESULTS

The MIC was taken as the lowest concentration of antifungal agent that substantially inhibit growth of the organism as detected visually .For the conventional microdilution procedure ,the growth in each MIC well is compared with that of the growth control with the aid of reading mirror .Each micro titer well was then given a numerical score as follows(54)

4 - No reduction in growth

3 – Slight reduction in growth or approximately 80% of growth control (drug free medium)

2 – Prominent reduction in growth or approximately 50% of growth control

1 – Slight growth or approximately 25% of growth control

0 – optically clear or absence of growth. (NCCLS M-38A)

MIC results recorded in mg/ml

DRUG	**END POINT FOR MIC**
Itraconazole (100%)-(80)	Score "0"
Fluconazole, Ketoconazole	Score "2" or less

OBSERVATION AND RESULTS

TABLE – 1

ANALYSIS OF GENDER RATIO

GENDER	FREQUENCY	%
Female	78	45.9%
Male	92	54.1%
TOTAL	170	100%

Out of 170 samples taken from patients 78(45.9%) were females and 92(54.1%) were males.

TABLE - 2

AGE DISTRIBUTION OF PATIENTS IN THE STUDY

AGE	NUMBER	PERCENTAGE
>10	5	2.9
11-20	32	18.8
21-30	30	17.6
31-40	29	17.1
41-50	35	20.6
51-60	27	15.9
61-70	12	7.1

Out of 170 patients , maximum cases reported were above 20 yrs (70%)and the least were from below 10yrs(2.9%). In the age group of 41-50 20.6% were seen.

TABLE - 3

PERCENTAGE CORRELATION OF THE CASES WITH HISTORY

S.NO	HISTORY	TOTAL NO. OF CASE	%
1	Fresh cases	82	48.2%
2	Past episodes	24	14.1%
3	Contact with diseased	10	5.8%
4	Contact with animals	18	10.5%
5	Treatment discontinued	36	21.1%

Out of 170 patients from whom the specimens were collected, 82 (48.2%) cases were fresh cases.36 (21.1%) of cases belonged to irregular treatment category..24 (14.1%) were relapses. 10 (5.8%) patients had contact history with patients in house hold and work spot and 18(10.5%) with animals.

TABLE – 4

PERCENTAGE OF SPECIMENS FROM DIFFERENT SITES OF COLLECTION

TOTAL NO. OF SPECIMENS	SKIN SCRAPPINGS	NAIL CLIPPINGS	HAIR
170	75	75	20

Out of 170 specimens collected, 75 were skin scrapings, 75 were nail clippings and 20 were hair samples.

TABLE – 5

CLINICAL TYPES OF DERMATOPHYTOSIS

SPECIMEN COLLECTION	CLINICAL TYPES OF LESIONS	NO. OF CASE	%
SKIN SCRAPPINGS (n=75)	Tinea corporis	49	65.3%
	Tinea cruris	19	25.3%
	Tinea mannum	4	5.3%
	Tinea pedis	3	4%
HAIR (n=20)	Tinea capitis	18	90%
	Tinea barbae	2	10%
NAIL CLIPPING (n=75)	Tinea unguium	75	100%

From skin(75), Tinea corporis was the major clinical presentation 49(65.3%) in this study.Tinea pedis was seen in 3(4%) of cases.

From hair (21), 19 (90%) were from Tinea capitis,2 (10%) were from Tinea barbae.**From nail clipping(n=75)** 21(100%) were from Tinea unguium.

TABLE – 6

PERCENTAGE OF ISOLATES FROM TOTAL SPECIMEN COLLECTED

TOTAL NO. OF CASES	NO. OF DERMATOPHYTES ISOLATED	OTHERS	NO GROWTH
170	60 35.2%	62 36.4%	48 26.9%

Out of 170 specimens, 60(35.2%) dermatophytes were isolated. There was 62(36.4%) other fungi grown which included non dermatophytes and contaminants. There was no growth found in 48 specimens (26.9%)

TABLE – 7

ANALYSIS OF MYCOLOGICAL CONFIRMITY BY CULTURE AND KOH WET MOUNT

S.NO	LESION	KOH WET MOUNT +VE CULTURE +VE	KOH WET MOUNT -VE CULTURE +VE	KOH WET MOUNT +VE CULTURE – VE
1	Tinea corporis (n=15)	12 80%	3 20%	2 13.3%
2	Tinea cruris (n=8)	6 75%	2 25%	3 37.5%
3	Tinea capitis (n=7)	4 57.1%	3 42.8%	1 14.2%
4	Tinea mannum (n=4)	3 75%	1 25%	2 50%
5	Tinea pedis (n=3)	2 66.6%	1 33.3%	0
6	Tinea barbae (n=2)	1 50%	1 50%	0
7	Tinea unguium (n=21)	14 66.6%	7 33,3%	5 23.8%
TOTAL	60	42 (70%)	18 (30%)	13 (7.6%)

Of the 60 culture positive isolates, 42 (70%) were KOH wet mount positive and culture positive, 18 were KOH negative and culture positive and 13 were KOH positive and culture negative.

TABLE - 8

CULTURE POSITIVE ISOLATES(n=60)

GENUS	SPECIES	NO. OF ISOLATES	%	TOTAL	TOTAL %
Trichophyton	rubrum	16	26.6%	55	93.9%
	mentagrophytes	13	21.6%		
	tonsurans	10	16%		
	verrrucosum	8	13%		
	violaceum	6	10%		
	schoenleinii	2	3.3%		
Epidermophyton	floccosum	2	3.3%	2	3.3%
Microsporum	gypseum	2	3.3%	3	5%
	audouinii	1	1.6%		

Out of 60 isolates of dermatophytes

55.(93.9%) isolates belonged to the Trichophyton spp.of which T.rubrum was the predominant isolate 16 (26.6%) followed by T.mentagrophytes 13 isolates (21.6%), T.tonsurans 10 (16%) isolates T.verrucosum 8 isolates (13%), T.violaceum 6 isolates (10%) and T.schoenleinii 2 isolates (3.3%).3 (5%) isolates belongs to Microsporum spp. Of which M. gypseum was 2 isolates (3.3%) and one (1.6%).isolate was M. audouinii.1 isolate belonged to Epidermophyton floccosum (3.3%).

TABLE – 9

AGE AND SEX DISTRIBUTION IN DIFFERENT SPP. ISOLATED

AGE	0-10		11-20		21-30		31-40		41-50		51-60		61-70	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
T.rubrum			3		4				3	1	2	1	2	
T.mentagrophytes			1		2	3		2	3			1		1
T.tonsurans			3		1		2	1	1		1	1		
T.verrucosum	1		3			1				1	1		1	
T.violaceum	2		1	1				1	1					
T.shoenleinii				1		1								
M.gypseum			1								1			
M.audouini					1									
E.floccosum			1								1			
TOTAL	3	0	13	2	8	5	2	4	8	2	6	3	3	1

T.rubrum was isolated predominantly in adults (13) that too in males (11) than in females (2). Females showed a higher incidence with T.mentagrophytes infection (7) than males (6).

TABLE - 10

DERMATOPHYTES SPP ISOLATED FROM THE SKIN (n=28)

DERMATOPHYTES Spp	Tinea corporis	Tinea cruris	Tinea mannum	Tinea Pedis	Total	%
T.rubrum	7	**1**	**1**	**1**	**10**	**35.7%**
T.mentagrophytes	4	**1**	**2**	**-**	**7**	**25%**
T.tonsurans	2	**-**	**1**	**2**	**5**	**17.8%**
T.verrucosum	-	**2**	**-**	**-**	**2**	**7.1%**
E.floccosum	-	**2**	**-**	**-**	**2**	**7.1%**
M.gypseum	2	**-**	**-**	**-**	**2**	**7.1%**
	15	**6**	**4**	**3**	**28**	

In Tinea corporis, T.rubrum was the predominant isolate 7/16(35.7%), followed by T.mentagrophytes 4/16(25%), T.tonsurans (12.5%) and M.gypseum 2/16(12.5%). In T. cruris, isolates of 2(33.3%) each of T. verrucosum and E.floccosum, 1(16.6%) Out of 4 dermatophytes isolated from Tinea manum , 2 were from T. mentagrophytes and 2 each of T. rubrum and T. tonsurans.Out of 3 dermatophytes isolated from Tinea pedis, 2 were T. tonsurans and 1was T rubrum. Out of 2 dermatopytes isolated, one each of T.mentagrophytes and T. verrucosum were isolated.

TABLE - 11
DERMATOPHYTES SPP ISOLATED FROM THE NAIL (n=21)

DERMATOPHYTES Spp	NO. OF ISOLATES	%
T.rubrum	6	28.5%
T.mentagrophytes	5	23.8%
T.tonsurans	2	9.5%
T.verrucosum	4	19%
T.violateum	2	9.5%
T.schonlienii	2	9.5%

Out of the 21 isolated dermatophytes in Tinea unguium,T. rubrum was the predominant isolate of 6 in number, followed by T. mentagrophytes 5 in number. There was 2 isolates of T. tonsurans ,4 isolates were T.verrucosum, 2 isolates were T. violateum and 2 isolates were T. schoenlinii.

TABLE -12
DERMATOPHYTES SPP ISOLATED FROM THE HAIR (n=11)

DERMATOPHYTES Spp	TINEA CAPITIS	TINEA BARBAE	TOTAL	%
T.menagrophytes	-	1	1	9%
T.tonsurans	3		3	11.1
T.verrucosum	1	1	2	27.2%
T.violateum	4	-	4	36.3%
M.audounii	1	-	1	9%

Out of 11 dermatopytes isolated of Tinea capitis , 4 were T.violaceum and 4 were T.tonsurans, T. verrucosum and M. audoiuni. 2isolates from T.barbae, one each of T.mentagrophytes and T.verrucosum.

ANTIFUNGAL SUSCEPTIBILITY TESTING BY AGAR DILUTION METHOD

TABLE - 13

DRUG –GRISEOFULVIN

SPECIES	DRUG CONCENTRATIONS(in µg/ml)											
	0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**		
T.rubrum (n=16)	0	3 18.7%	0	2 12.5%	2 12.5%	9 56.2%	-	-	-	-	0.5	1
T.mentagrophytes (n=13)	0	0	2 15.3%	2 15.3%	6 46.1%	3 23%	-	-	-	-	0.5	1
T.tonsurans (n=10)	0	3 30%	5 50%	2 20%		-	-	-	-	-	0.12	0.25
T.verrucosum (n=8)	0	0	2 25%	2 25%	2 25%	2 25%	-	-	-	-	0.25	1
T.violaceum (n=6)	0	0	1 16.6%	2 33.3%	2 33.3%	1 16.6%	-	-	-	-	0.5	1
T.schoenleinii (n=2)	0	0	1 50%	0	1 50%	-	-	-	-	-	0.12	0.5
E.floccosum (n=2)	0	0	1 50%	1 50%	-	-	-	-	-	-	0.12	0.25
M.gypseum (n=2)	0	0	0	1 50%	0	1 50%	-	-	-	-	0.25	1
M.audouinii (n=1)	0	0	0	1 100%	-	-	-	-	-	-		0.25

MIC 50 and MIC 90 of Griseofulvin for all the isolates of this study are as follows-T rubrum -0.5 and 1µg/ml respectively

T .mentagrophytes -0.5 and 1µg/ml respectively

T .tonsurans -0.12 and 0.25µg/ml respectively

T.verrucosum -0.25 and 1µg/ml respectively

T.violaceum -0.5 and 1µg/ml respectively

T.schoenleinii -0.12 and 0.5µg/ml respectively

E.floccosum -0.12 and 0.25µg/ml respectively

M.gypseum -0.25 and 1µg/ml respectively

M.audouinii- 0.25 respectively(MIC 90)

TABLE -14

DRUG KETOCONAZOLE

SPECIES	DRUG CONCENTRATIONS(in µg/ml)											
	0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**MIC50**	**MIC90**
T.rubrum (n=16)	0	0	7 43.7%	2 12.5%	3 18.7%	4 25%	-	-	-	-	0.12	1
T.mentagrophytes (n=13)	0	1 7.6%	1 7.6%	2 15.3%	5 38.4%	4 30.7%	-	-	-	-	0.5	1
T.tonsurans (n=10)	0	0	2 20%	2 20%	5 50%	1 10%	-	-	-	-	0.5	1
T.verrucosum (n=8)	0	0	2 25%	1 12.5%	2 25%	3 37.5%	-	-	-	-	0.5	1
T.violaceum (n=6)	0	0	0	0	3 50%	3 50%	-	-	-	-	0.5	1
T.schoenleinii (n=2)	0	0	1 50%	0	1 50%	-	-	-	-	-	0.12	0.5
E.floccosum (n=2)	0	0	0	1 50%	0	1 50%	-	-	-	-	0.25	1
M.gypseum (n=2)	0	0	1 50%	0	1 50%	-	-	-	-	-	0.12	0.5
M.audouinii (n=1)	0	0	0	0	1 100%	-	-	-	-	-		0.5

MIC 50 and MIC 90 of Ketoconazole for all the isolates of this study are as follows-T rubrum -0.12 and 1µg/ml / 0.12 and 0.5µg/m respectively

T .mentagrophytes -0.5 and 1µg/ml respectively

T .tonsurans -0.05 and 1µg/ml respectively

T.verrucosum -0.5 and 1µg/ml respectively

T.violaceum -0.5 and 1µg/ml respectively

T.schoenleinii -0.12 and 0.5µg/ml respectively

E.floccosum -0.25 and 1µg/ml respectively

M.gypseum -0.12 and 0.5µg/ml respectively

M.audouinii- 0.5 µg/ml (MIC 90)

TABLE – 15

DRUG FLUCONAZOLE

SPECIES	DRUG CONCENATIONS(in µg/ml)											
	0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**MIC50**	**MIC90**
T.rubrum (n=16)	0	0	0	5 31.2%	5 31.2%	3 18.7%	3 18.7%	-	-	-	2	8
T.mentagrophytes (n==13)	0	0	0	0	0	3 23%	2 15.3%	3 23%	5 38.4%	-	16	32
T.tonsurans (n=10)	0	0	0	0	0	3 30%	3 30%	4 40%	-	-	8	16
T.verrucosum (n=8)	0	0	0	0	0	0	2 25%	5 62.5%	2 25%	-	16	32
T.violaceum (n=6)	0	0	0	0	2 33.3%	1 16.6%	2 33.3%	1 16.6%	-	-	4	16
T.schoenleinii (n=2)	0	0	0	0	0	0	1 50%	-	1 50%	-	8	32
E.floccosum (n=2)	0	0	0	0	0	1 50%	0	1 50%	-	-	4	16
M.gypseum (n=2)	0	0	0	0	0	0	1 50%	-	1 50%	-	8	32
M.audouinii (n=1)	0	0	0	0	0	0	-	1 100%	-	-		16

MIC 50 and MIC 90 of Fluconazole for all the isolates of this study are as follows :

T rubrum -0.2 and 8µg/ml respectively

T .mentagrophytes -16 and 32µg/ml respectively

T .tonsurans -8 and 16µg/ml respectively

T.verrucosum -16 and 32µg/ml respectively

T.violaceum -4 and 16µg/ml respectively

T.schoenleinii -8 and 32µg/ml respectively

E.floccosum -4 and 16µg/ml respectively

M.gypseum -8 and 32µg/ml respectively

M.audouinii- 16 µg/ml (MIC 90)

TABLE – 16

DRUG – ITRACONAZOLE

SPECIES	ITRACONAZOLE -DRUG CONCENTRATIONS(in µg/ml)											
	0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**MIC 50**	**MIC 90**
T.rubrum (n=16)	0	0	2 6.25%	6 37.5%	5 31.2%	3 18.7%	-	-	-	-	0.5	1
T.mentagrophytes (n=13)	0	2 15.3%	4 30.7%	3 23%	4 30.7%	-	-	-	-	-	0.12	0.5
T.tonsurans (n=10)	0	1 10%	1 10%	0	3 30%	0	5 50%	-	-	-	0.5	2
T.verrucosum (n=8)	0	0	4 50%	0	2 25%	0	2 25%	-	-	-	0.12	2
T.violaceum (n=6)	0	0	0	1 16.6%	5 83.4%	-	-	-	-	-	0	0.5
T.schoenleinii (n=2)	0	0	0	1 50%	0	0	1 50%	-	-	-	0.5	2
E.floccosum (n=2)	0	0	0	0	1 50%	1 50%	-	-	-	-	0.5	1
M.gypseum (n=2)	0	0	1 50%	0	0	0	0	1 50%	-	-	0.12	4
M.audouinii (n=1)	0	0	0	0	0	1 100%	-	-	-	-		1

MIC 50 and MIC 90 of Itraconazole for all the isolates of this study are as follows-T rubrum -0.5 and 1µg/ml respectively.

T .mentagrophytes -0.12 and 0.5µg/ml respectively

T .tonsurans -0.5 and 2µg/ml.03 respectively

T.verrucosum -0.12 and 2µg/ml respectively

T.violaceum -0 and 0.5µg/ml respectively

T.schoenleinii -0.5 and 2µg/ml respectively

E.floccosum -0.5 and 1µg/ml respectively

M.gypseum -0.12 and 4µg/ml respectively

M.audouinii- 1 respectively(MIC 90)

TABLE – 17

DRUG - TERBINIFINE

SPECIES	DRUG CONCENTRATIONS (in µg/ml)											
	0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**MIC 50**	**MIC 90**
T.rubrum (n=16)	2 12.5%	5 31.2%	9 56.2%	-	-	-	-	-	-	-	0.06	0.12
T.mentagrophytes (n=13)	2 15.3%	5 35.7%	6 42.8%	-	-	-	-	-	-	-	0.06	0.12
T.tonsurans (n=10)	0	7 70%	3 30%	-	-	-	-	-	-	-	0.06	0.12
T.verrucosum (n=8)	4 50%	2 25%	2 25%	-	-	-	-	-	-	-	0.03	0.12
T.violaceum (n=6)	0	3 50%	3 50%	-	-	-	-	-	-	-	0.06	0.12
T.schoenleinii (n=2)	0	0	2 100%	-	-	-	-	-	-	-	0	0.12
E.floccosum (n=2)	2 100%	-	-	-	-	-	-	-	-	-	0	0.03
M.gypseum (n=2)	0	0	2 100%	-	-	-	-	-	-	-	0	0.12
M.audouinii (n=1)	0	1 100%	-	-	-	-	-	-	-	-		0.06

MIC 50 and MIC 90 of Terbinafine for all the isolates of this study are as follows-T rubrum -0.06 and 0.12µg/ml respectively

T .mentagrophytes -0.06 and 0.12µg/ml respectively

T .tonsurans -0.06 and 0.12µg/ml respectively

T.verrucosum -0.03 and 0.12µg/ml respectively

T.violaceum -0.06 and 0.12µg/ml respectively

T.schoenleinii -0 and 0.12µg/ml respectively

E.floccosum -0 and 0.25µg/ml respectively

M.gypseum -0 and 0.12µg/ml respectively

M.audouinii- 0.06 µg/ml (MIC 90)

ANTIFUNGAL SUSCEPTIBILITY TESTING BY MICROBROTH DILUTION METHOD

TABLE 18

DRUG – GRISOFULVIN

SPECIES	DRUG CONCENTRATIONS (in µg/ml)											
	0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**MIC 50**	**MIC 90**
T.rubrum (n=16)	0	2 12.5%	6 37.5%	8 50%	-	-	-	-	-	-	0.12	0.25
T.mentagrophytes (n=13)	3 23%	4 30.7%	0	6 46%	-	-	-	-	-	-	0.06	0.25
T.tonsurans (n=10)	3 30%	4 40%	3 30%	-	-	-	-	-	-	-	0.06	0.12
T.verrucosum (n=8)	3 37.5%	2 25%	0	3 37.5%	-	-	-	-	-	-	0.06	0.25
T.violaceum (n=6)	0	3 50%	3 50%	-	-	-	-	-	-	-	0.06	0.12
T.schoenleinii (n=2)	0	1 50%	0	1 50%	-	-	-	-	-	-	0.06	0.25
E.floccosum (n=2)	1 50%	0	0	1 50%	-	-	-	-	-	-	0.03	0.25
M.gypseum (n=2)	0	2 100%	-	-	-	-	-	-	-	-	0	0.06
M.audouinii (n=1)	0	0	1 100%	-	-	-	-	-	-	-	0	0.12

MIC 50 and MIC 90 of Griseofulvin for all the isolates of this study are as follows-T rubrum - 0.12 and 0.25µg/ml respectively

T .mentagrophytes -0.06 and 0.25µg/ml respectively

T .tonsurans -0.06 and 0.12µg/ml respectively

T.verrucosum -0.06 and 0.12µg/ml respectively

T.violaceum -0.06 and 0.12µg/ml respectively

T.schoenleinii -0.06 and 0.25µg/ml respectively

E.floccosum -0.03 and 0.25µg/ml respectively

M.gypseum - 0 and 0.06µg/ml respectively

M.audouinii- 0.12µg/ml(MIC 90)

TABLE – 19

DRUG – KETOCONAZOLE

SPECIES	DRUG CONCENTRATIONS (in µg/ml)											
	0.03	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**MIC 50**	**MIC 90**
T.rubrum (n=16)	0	2 12.5%	7 43.7%	4 25%	3 18.7%	-	-	-	-	-	0.12	0.5
T.mentagrophyte (n=13)	3 23%	0	5 35.7%	5 35.7%	-	-	-	-	-	-	0.12	0.25
T.tonsurans (n=10)	0	6 60%	2 20%	2 20%	-	-	-	-	-	-	0.06	0.25
T.verrucosum (n=8)	0	3 37.5%	1 12.5%	0	4 50%	-	-	-	-	-	0.12	0.5
T.violaceum (n=6)	2 33.3%	0	4 66.6%	-	-	-	-	-	-	-	0.03	0.12
T.schoenleinii (n=2)	0	0	1 50%	1 50%	-	-	-	-	-	-	0.12	0.25
E.floccosum (n=2)	1 50%	0	0	0	1 50%	-	-	-	-	-	0.03	0.5
M.gypseum (n=2)	0	0	1 50%	0	1 50%	-	-	-	-	-	0.12	0.5
M.audouinii (n=1)	0	0	0	0	1 100%	-	-	-	-	-		0.5

MIC 50 and MIC 90 of Ketoconazole for all the isolates of this study are as follows-T rubrum -0.12 and 0.5µg/ml respectively

T .mentagrophytes -0.12 and 0.25µg/ml respectively

T .tonsurans -0.06 and 0.25µg/ml respectively

T.verrucosum -0.12 and 0.5µg/ml respectively

T.violaceum -0.03 and 0.12µg/ml respectively

T.schoenleinii -0.12 and 0.25µg/ml respectively

E.floccosum -0.03 and 0.5µg/ml respectively

M.gypseum -0.12 and 0.5µg/ml respectively

M.audouinii- 0.5µg/ml (MIC 90)

TABLE – 20

DRUG – FLUCONAZOLE

SPECIES	DRUG CONCENTRATIONS (in µg/ml)											
	0.06	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**Mic 50**	**Mic 90**
T.rubrum (n=16)	0	0	0	0	8 50%	5 31.2%	3 18.7%	-	-	-	1	4
T.mentagrophytes (n=13)	0	0	0	0	0	7 53.8%	2 15.3%	4 30.7%	-	-	2	8
T.tonsurans (n=10)	0	0	0	0	0	5 50%	3 30%	2 20%	-	-	2	8
T.verrucosum (n=8)	0	0	0	0	0	4 50%	4 50%	-	-	-	2	4
T.violaceum (n=6)	0	0	0	0	0	0	5 83.3%	-	1 16.6%	-	4	16
T.schoenleinii (n=2)	0	0	0	0	0	1 50%	-	-	1 50%	-	2	16
E.floccosum (n=2)	0	0	0	0	0	0	2 100%	-	-	-	0	4
M.gypseum (n=2)	0	0	0	0	0	0	1 50%	-	-	1 50%	4	32
M.audouinii (n=1)	0	0	0	0	0	0	1 100%	-	-	-		4

MIC 50 and MIC 90 of Fluconazole for all the isolates of this study are as follows-T rubrum -1 and 4µg/ml respectively

T .mentagrophytes -2 and 8µg/ml respectively

T .tonsurans -2 and 8µg/ml respectively

T.verrucosum -2 and 4µg/ml respectively

T.violaceum -4 and 16µg/ml respectively

T.schoenleinii -2 and 16µg/ml respectively

E.floccosum -0 and 4µg/ml respectively

M.gypseum -4 and 32µg/ml respectively

M.audouinii- 4µg/ml (MIC 90)

TABLE – 21

DRUG – ITRACONAZOLE

SPECIES	DRUG CONCENTRATIONS (in µg/ml)											
	0.0075	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**Mic 50**	**Mic 90**
T.rubrum (n=16)	0	4 25%	1 6.25%	4 25%	4 25%	3 18.7%	-	-	-	-	0.06	0.25
T.mentagrophytes (n=13)	2 15.3%	5 35.7%	0	2 15.3%	1 7.6%	-	-	-	-	-	0.01 5	0.12
T.tonsurans (n=10)	0	1 10%	4 40%	3 30%	0	2 20%	-	-	-	-	0.03	0.25
T.verrucosum (n=8)	0	1 12.5%	1 12.5%	4 50%	0	2 25%	-	-	-	-	0.06	0.25
T.violaceum (n=6)	0	0	5 62.5%	1 16.6%	-	-	-	-	-	-	0.03	0.12
T.schoenleinii (n=2)	0	0	1 50%	0	1 50%	-	-	-	-	-	0.03	0.12
E.floccosum (n=2)	0	0	1 50%	1 50%	-	-	-	-	-	-	0.03	0.06
M.gypseum (n=2)	0	1 50%	0	1 50%	-	-	-	-	-	-	0.01 5	0.06
M.audouinii (n=1)	0	0	1 100%	-	-	-	-	-	-	-		0.03

MIC 50 and MIC 90 of Itraconazole for all the isolates of this study are as follows-T rubrum -0.06 and 0.25µg / ml respectively

T .mentagrophytes -0.015 and 0.12µg / ml respectively

T .tonsurans -0.03 and 0.25µg / ml respectively

T.verrucosum -0.06 and 0.25µg / ml respectively

T.violaceum -0.03 and 0.12µg / ml respectively

T.schoenleinii -0.03 and 0.12µg / ml respectively

E.floccosum -0.03 and 0.06µg / ml respectively

M.gypseum -0.015 and 0.06µg / ml respectively

M.audouinii- 0.03µg/ml (MIC 90)

TABLE – 22

DRUG – TERBINAFINE

SPECIES	DRUG CONCENTRATIONS (in µg/ml)											
	0.007	0.015	0.03	0.06	0.12	0.25	O.5	1	2	4	MIC 50	MIC 90
T.rubrum (n=16)	3 18.7%	3 18.7%	5 31.2%	5 31.7%	-	-	-	-	-	-	0.03	0.06
T.mentagrophytes (n=13)	3 23%	5 35.7%	3 23%	2 15.3%	-	-	-	-	-	-	0.015	0.06
T.tonsurans (n=10)	0	0	6 60%	4 40%	-	-	-	-	-	-	0.03	0.06
T.verrucosum (n=8)	3 37.5	1 12.5%	2 25%	2 25%	-	-	-	-	-	-	0.015	0.06
T.violaceum (n=6)	3 50%	2 33.3%	0	1 16.6%	-	-	-	-	-	-	0.007 5	0.06
T.schoenleinii (n=2)	0	2 100%	-	-	-	-	-	-	-	-		0.015
E.floccosum (n=2)	1 50%	0	0	1 50%		-	-	-	-	-	0.007 5	0.06
M.gypseum (n=2)	0	1 50%	1 50%	-	-	-	-	-	-	-	0.015	0.03
M.audouinii (n=1)	0	0	1 100%	0	-	-	-	-	-	-		0.03

MIC 50 and MIC 90 of Terbinafine for all the isolates of this study are as follows-T rubrum -0.03 and 0.12µg/ml respectively

T .mentagrophytes -0.015 and 0.12µg/ml respectively

T .tonsurans -0.03 and 0.12µg/ml respectively

T.verrucosum -0.015 and 0.06µg/ml respectively

T.violaceum -0.007 and 0.06µg/ml respectively

T.schoenleinii -0 and 0.015µg/ml respectively

E.floccosum -0.007 and 0.12µg/ml respectively

M.gypseum -0.015 and 0.25µg/ml respectively

M.audouinii- 0.12µg/ml (MIC 90)

DISCUSSION

Out of 170 samples taken from patients 78(45.9%) were females and 92(54.1%) were males . Farheen Ansari et al 2006 showed that males were found to be affected more than females. The lower incidence in females may due to prevailing social stigma. Male preponderance may be correlated with the occupational hazards related to thier nature of work , the frequent inter action with different people of the society, environmental conditions such as hot and humid weather, poor personal hygiene and illiteracy are other major factors that influence dermatophytosis in this part of the country as observed by Venkatesh et al, Padhye et al, Kamalam A, Thambiah AS(1976); Ranganathan et al (1995)

In this study dematophytosis was more common above 20 years(70%). Incidence of dermatophytosis is more common above the age group of 20 yrs as shown in the studies of Gupta BK et al , 1993-(77.9%) and Murdia P et al ,1987 (53.3%).

Out of the 170 cases 82 (48.2%) cases were fresh cases who did not have the disease earlier or had associates with skin infections. 24 (14.1%) patients had similar past history skin lesions for which they were treated.18 (10.5%) patients had contact with animals. The living condition of patients played a important role as shown in the study conducted by G. Venkatesan et al (2006). Some of the patients had close contact with domestic / pet animals such as cattle, dogs and cats.10 (5.8%) patients had contact history with patients in house hold, school ,work and animals. Epidemilogical evidence indicates that T. violateum , T. schoenleinii and Epidermophyton floccosum can be transmitted through sharing towels and garments.

A total number of 170 samples were collected , skin -75, nail -75 and 20 hair samples. The global incidence of Onycomycosis is increasing and it continues to spread and persist as shown by studies of Illikit M et al (Turkey), Koursideu et al (Northern Greece), Garg A et al(Central India), Drake LA et al and Elewski BE et al(Northeastern Ohio).

In this study Tinea corporis was 49/170 (28.8%) cases followed by tinea cruris 19/170 (11.1%) as seen in study conducted by Suman and Beena ,2003 -58.84% Tinea corporis and 12.3% of Tinea cruris In their studies observed that most of the patients were involved in exhausting physical work with profuse sweating . Furthermore they wear tight synthetic clothes resulting in conditions like increased dampness and warmth of the body facilitating the skin surface suitable for the growth of dermatophytes.

Tinea capitis was detected in 20(11.7%) cases in the present study as against the study conducted in Karachi by Farheen Ansari et al 2006 where tinea capitis and tinea corporis were the most prevailing dermatophytic infection affecting males and children .

A low incidence of tinea pedis 3(4%) was seen in the present study as in the study of Venkatesan et al 4(5.6%)

In the present study, Tinae unguium was seen in 75 (44%) in accordance with a study conducted by Kaur et al 2007 where 45% was recorded. Because of the limited number of large-scale studies in India , the base line incidence of onycomycosis is not firmly established , as highlighted by Veer P et al , Ravinder K et al and Dogra S et al.

In KOH examination of the samples, 42(70%)were both KOH positive and culture positive.. This in similarity to studies conducted by Metha et al (1997) and Lalithammal et al were higher-72% and 71.6%.

13(7.6%) were KOH positive and culture negative. Baskaran et al" s study shows 8.89% KOH positive and culture negative which in similarity with the present study (7.6%)

In the present study, 60(35.2%) were culture positive for dermatophytes. In studies conducted by Kaviarasan PK et al, BindhuV et al and Ellabib MS et al the isolation rate was higher, ranging from 45.3 – 52.7%.

Out of 60 isolates of dermatophytes 55 (93.9%) isolates belonged to the Trichophyton spp.of which T.rubrum was the predominant isolate 16 (26.6%) followed by T.mentagrophytes 13 (21.6%) . In Kannan et al studies T. rubrum was the main isolate(81%) This coincides with most of the earlier works of Pandey et al ,1970; Verenker et al, 1991;Summana and Singaracharya ,2004

Mentagrophytes was the second common isolate from the body site 13/60 (21.6%). This is in conformity with studies of Urmil Mohan et al, were the isolation rate was 28.2% Mehta JP et al and Nagakatti PS et al. have also recorded a isolation rate of 22% and 29%.

3 (5%) isolates belongs to Microsporum spp. Of which M. gypseum was 2 isolates (3.3%) and one isolate was M. audouinii(1.6%).1 isolate belonged to Epidermophyton floccosum (3.3%). The members of the genera of Epidermophyton 2/60 (3.3%)and Microsporum 3/60(4.9%) accounted

for lower percentage of infection when compared to trichophyton species as in Kannan et al , 2006 studies 2/53(3.7%).

Adults had a higher susceptibility to T.rubrum infection (85.25%) than children(18.7%) which is consistent with the observations of Shahindokht Bassiri et, Desai and Bhat et al and Ng et al

In the present study, 28 dermatophytes were isolated from skin scales of Tinea corporis, Tinea cruris, Tinea mannum and Tinea pedis. T.rubrum was the predominant isolate 10 (35.7%) and second most commom isolate was T.mentagrophytes ,7 (25%) .In the study of Kannan et al- T.rubrum70%.and T.mentagrophytes was16.7%.

In this study , E.floccosum were isolated in 2 each (33.3% each) of cases out of 6 isolates from Tinea cruris. In a epidemiological survey of dermatophytosis in Tehran 2000 to 2005 Shahindokht Bassiri et al have found that Epiermophyton floccosum 85% remained the most prevalent fungal pathogen and increased incidence of this species in cases of Tinea cruris.

Out of 9 isolates from Tinea capitis 44.1% isolates were T.violateum,33.3% were T.tonsurans and 11.1% of isolates were T.adouinii and T.verrucosum. T.violaceum remains the most predominant isolate as in studies of Farheen Ansari with a isolate rate of 41%as in other studies of Falahati et al (2003);Jahromi SB et al 2006 and Lari AR et al 2005. Kamalam and Thambiah (1979) studied tinea capitis in two schools In the first school T, violateum was isolated in 6.22% of children `and the lesions were inflammatory. In the other, T.violateum was isolated in 59.75% of children `and the lesions were non inflammatory. Sentamil et al have

shown that T.violeceum is the commest isolate from cases of Tinea capitis in Tamil nadu India.

Out of the 21 dermatophytes isolated from Tinea unguium,,6 **(** 28.3%) of the isolates were T. rubrum, 5(23.8%) were T.mentagrophytes and T.verrucosum 4(19%) which almost coincides with the study of Muditha Guptha et al were T.rubrum16(32.6%) ,T.mentagrophytes 3(6.1%) and T.verrucosum 1(2.1%) were isolated. These agents are the common species infecting the nail as documented earlier by Smitha Sarma et al in a study conducted in 2004-2007 from north India, in their study T.rubrum (47%) was the most common isolate, followed by T.mentagrophytes (20.4%) .but here T.tonsurans was third predominant isolate(11.7%).

ANTIFUNGAL SUSCEPTIBILITY TESTING (AGAR DILUTION AND MICRODILUTION METHODS) IN VITRO ACTIVITIES OF 5 ANTIFUNGAL DRUGS BY AGAR DILUTION METHOD

DERMATO-PHYTES	MIC (µg/ml)	DRUGS (µg/ml)				
		GRISEO-FULVIN	KETOCO NAZOLE	FLUCONA Z0LE	ITRACONAZ OLE	TERBINAFINE
T.rubrum	Range	0.06-1	0.12-0.5	1-8	012-1	0.03-0.12
	MIC50	0.5	0.12	2	0.5	0.06
	MIC90	1	1	8	1	0.12
T.mentagrophytes	Range	0.12-1	0.06-1	4-32	0.06-0.5	0.03-0.12
	MIC50	0.5	0.5	16	0.12	0.06
	MIC90	1	1	32	0.5	0.12
T.tonsurans	Range	0.06-0.5	0.12-1	4-16	0.06-2	0.06-0.12
	MIC50	0.12	0.5	8	0.5	0.06
	MIC90	0.25	1	16	2	0.12
T.verrucosum	Range	0.12-0.5	0.12-1	8-32	0.12-2	0.03-0.12
	MIC50	0.5	0.5	16	0.12	0.03
	MIC90	1	1	32	2	0.12
T.violaceum	Range	0.12-1	0.5-1	2-16	025-0.5	0.06-0.12
	MIC50	0.5	0.5	4	0	0.06
	MIC90	1	1	16	0.5	0.12
T.schoenleinii	Range	0.12-0.5	0.12-0.5	8-32	0.5-2	0.12
	MIC50	0.12	0.12	8	0.5	0
	MIC90	0.5	0.5	32	2	0.12
M.gypseum	Range	0.12-0.5	0.12-05	8-32	0.12-4	0.12
	MIC50	0.25	0.12	8	0.12	0
	MIC90	1	0.5	32	4	0.12
M.audouinii	Range	0.25	0.5	16	1	0.06
	MIC50	0	0	0	0	0
	MIC90	0.25	0.5	16	1	0.06
E.floccosum	Range	0.12-0.25	0.25-1	4-16	0.5-1	0.25
	MIC50	0.12	0.25	4	0.5	0
	MIC90	0.25	1	16	1	0.25
All dermatophytes	Range	0.06-0.1	0.06-1	1-32	0.06-4	0.03-0.12
	MIC50	0.25	0.5	8	0.5	0.06
	MIC90	1	1	16	1	0.12

The drugs taken for antifungal susceptibility testing were Griseofulvin, ketoconazole, Fluconazole, Itraconazole and Terbinafine. Antifungal susceptibility testing was done by agar dilution and microbroth dilution methods.

COMPARISON OF INVITRO ACTIVITIES OF 5 ANTI FUNGAL DRUGS BY AGAR DILUTION METHOD

	Drugs	Present study			Dr.PANKAJALAKSHMI'S STUDY		
		MIC Range (µg/ml)	MIC 50 (µg/ml)	MIC 90 (µg/ml)	MIC Range (µg/ml)	MIC 50 (µg/ml)	MIC 90 (µg/ml)
All dermatophytes.	Griseofulvin	0.06-0.1	0.25	1	0.1-10	1	5
	Ketoconazole	0.06-0.1	0.5	1	0.01-5	1	2.5
	Fluconazole	1-32	8	16	-	-	-
	Itraconazole	0.06-4	0.5	1	0.01-0.5	0.1	0.5
	Terbinafine	0.03-0.12	0.06	0.12	0.001-0.01	0.01	0.1

In the present study The MIC range, MIC 50 and MIC 90 for the drug Grisoefulvin was found to be 0.06-0.1, 0.25 and 1 respectively. The MIC range, MIC 50 and MIC 90 for the drug Ketoconazole was found to be 0.06-0.1, 0.5 and 1 respectively. The MIC range, MIC 50 and MIC 90 for the drug Fluconazole was found to be 1-32, 8 and 16 respectively. The MIC range, MIC 50 and MIC 90 for the drug Itraconazole was found to be 0.06-4, 0.5 and 1 respectively. The MIC range, MIC 50 and MIC 90 for the drug Terbinafine was found to be 0.03-0.12, 0.06 and 0.12 respectively.

In Dr. Pankajalaxsmi's study MIC range, MIC 50 and MIC 90 were found to be higher for Griseofulvin and Ketoconazole than the present study.the MIC range, MIC 50 and MIC 90 were equal for Itraconazole and lower for Terbinafine. Bertnard Favre et al (2003) conducted a comparison of invitro activities of 17 Antifungal drugs against a panel of the 20

dermatophytes by using a microdilution method have shown Griseofulvin, ketoconazole, Itraconazole and Terbinafine were the most potent agents.

IN VITRO ACTIVITIES OF 5 ANTIFUNGAL DRUGS BY MICRO BROTH DILUTION METHOD

DERMATO-PHYTES	DRUGS (µg/ml)					
	MIC (µg/ml)	GRISEO-FULVIN	KETOCO-NAZOLE	FLUCO-NAZ0LE	ITRACO-NAZOLE	TERBINAFINE
T.rubrum	Range MIC50 MIC90	0.06-0.12 0.12 0.25	0.06-0.5 0.12 0.5	1-4 1 4	0.015-0.25 0.06 0.25	0.03 0.12
T.mentagrophytes	Range MIC50 MIC90	0.06-0.25 0.06 0.25	0.12-0.25 0.12 0.25	2-8 2 8	0.007-0.12 0.015 0.12	0.015 0.12
T.tonsurans	Range MIC50 MIC90	0.03-0.12 0.06 0.12	0.06-0.25 0.06 0.25	2-8 2 8	0.015-0.25 0.03 0.12	0.03 0.12
T.verrucosum	Range MIC50 MIC90	0.03-0.25 0.03 0.25	0.06-0.5 0.06 0.5	2-4 2 4	0.015-0.25 0.06 0.25	0.015 0.06
T.violeceum	Range MIC50 MIC90	0.06-0.12 0.06 0.12	0.03-0.12 0.03 0.12	4-16 4 16	0.03-0.06 0.03 0.06	0.007 0.06
T.schoenleinii	Range MIC50 MIC90	0.06-0.25 0.06 0.25	0.12-0.25 0.12 0.25	2-16 2 16	0.03-0.12 0.03 0.12	0 0.015
M.gypseum	Range MIC50 MIC90	0.06-0.12 0.06 0.12	0.12-0.5 0.12 0.5	4-32 4 32	0.015-0.06 0.015 0.06	0.15 0.25
M.audouinii	Range MIC50 MIC90	0.12 0 0.12	0.5 0 0.5	4 0 4	0.13 0 0.03	0 0.12

COMPARISON OF INVITRO ACTIVITIES OF 5 ANTI FUNGAL BY MICRODILUTION METHOD

	Drugs	Present study			M.A.Ghannoum et al study (2004)		
		MIC Range (µg/ml)	MIC 50 (µg/ml)	MIC 90 (µg/ml)	MIC Range (µg/ml)	MIC 50 (µg/ml)	MIC 90 (µg/ml)
All dermatophytes.	Griseofulvin Ketoconazole Fluconazole Itraconazole Terbinafine	0.03-0.25 0.03-0.5 1-32 0.007-0.25 0.007-0.06	0.06 0.12 4 0.03 0.015	0.25 0.5 32 0.25 0.06	 0.12-64 0.12-16 0.001-0.05 0.001-0.5	 0.12 2 0.015 0.008	 0.5 16 0.125 0.03

In the present study The MIC range, MIC 50 and MIC 90 for the drug Grisoefulvin was found to be 0.03-0.25, 0.06 and 0.12 respectively. The MIC range, MIC 50 and MIC 90 for the drug Ketoconazole was found to be 0.03-0.5, 0.12 and 0.5 respectively. . The MIC range, MIC 50 and MIC 90

for the drug Fluconazole was found to be 1-32, and 1 respectively. The MIC range, MIC 50 and MIC 90 for the drug Itraconazole was found to be 0.007-0.06, 0.03- and 0.06 respectively. The MIC range, MIC 50 and MIC 90 for the drug Terbinafine was found to be 0.007-0.06, 0.015 and 0. respectively

The present study and the study conducted by M.A.Ghannoum et al in 2004 shows an increase in the MIC values of Griseofulvin and Fluconazole in the previous study. Similarities were seen in the other MIC values for Itra conazole and Terbinafine.

In a multicentre study performed by Espinel-Ingroff et al , found the lowest intra and interlaboratory aggrement for Itraconazole (59%-79% and 59% - 91%).

All the above results of this present study almost correlates with the previous studies conducted by B.Fernandez Torres et al,Bertrand Favre et al, M.A.Gannoum et al and I.Pujol et al.

In recent years several studies of in vitro susceptibility of dermatophytes have been done and the results have shown considerable variations. This variability is probably due to important methodological differences among the laboratories.

The microdilution assay for dermatophytes is convenient and reproducible. A marked reduction in the MIC values was seen in the microdilution method when compared to the agar dilution method in all drugs tested.

SUMMARY

170 samples from 170 patients affected by dermatophytosis were collected and processed.

Male / Female ratio was 54%: 46%.

Most of the cases (70%) were seen above 20 years . Tinea capitis was seen below 10 years.

16.3% of cases gave a history of contact with possible source of infection.

Of the 60 positive isolates, 70% were posive by KOH wet mount study and culture , 30% were KOH wet mount negative and culture positive and 7.6% were positive by KOH wet mount study and negative by culture. Culture positivity from skin scrapings were more in cases of Tinea corporis 65.3%, followed by Tinea cruris 25.3%, Tinea mannum 5.3% and Tinea pedis 3%.

The samples were skin scappings , hair and nail.60 cases were culture positive and out of these 93.9% belonged to Trichophyton species, 5% Microsporum species and 3.3% to Epidermophyton species

The predominant isolate from all samples were T.rubrum 26.6%. Adults were found to be more susceptible to T.rubrum infection than children.

In Tinea corporis, T.rubrum was isolated from 46.6% , from T.unguium it was 28.6%. T.mentagrophytes followed T.rubrum in isolation

rate 21.6%, highest 26.6% from T.corporis and 23.8% were from T.unguium.

In Tinea capitis the major isolate was T.violaceum 44.4%.

In tinea cruris, T.verrucosum 33.3% and E.floccosum 33.3% were the main isolates. All isolates of E.floccosum were from cases of Tinea cruris.

There were only two isolates of T.schoenleinii and both were from nail clippings.

Only one isolate was M.audouinii from hair sample.

Anti fungal susceptibility testing was performed by agar dilution method and microbroth dilution method for Griseofulvin, ketoconazole, fluconazole, Itraconazole and Terbinafine.

The MIC range for Griseofulvin by agar dilution method was 0.06µg/ml - 1µg/ml and by microbroth dilution method was 0.03 µg/ml – 0.25µg/ml.

The MIC range for ketoconazole by agar dilution method was 0.06µg/ml - 1µg/ml and by microbroth dilution method was 0.03 µg/ml – 0.5µg/ml

The MIC range for Fluconzole by agar dilution method was 1µg/ml - 32µg/ml and by microbroth dilution method was 0.5 µg/ml – 0.16µg/ml.

The MIC range for Itraconazole by agar dilution method was 0.06µg/ml - 4µg/ml and by microbroth dilution method was 0.007 µg/ml – 0.25µg/ml.

The MIC range for Terbinifine by agar dilution method was 0.03µg/ml – 0.12µg/ml and by microbroth dilution method was 0.007 µg/ml – 0.06µg/ml

The MIC range, MIC 50 and MIC 90 by both methods are as follows,

	Drugs	Agar dilution			Microbroth dilution		
		MIC Range (µg/ml)	**MIC 50 (µg/ml)**	**MIC 90 (µg/ml)**	**MIC Range (µg/ml)**	**MIC 50 (µg/ml)**	**MIC 90 (µg/ml)**
All dermatophytes.	Griseofulvin	0.06-0.1	0.25	1	0.03-0.25	0.06	0.12
	Ketoconazole	0.06-0.1	0.5	1	0.03-0.5	0.06	0.5
	Fluconazole	1-32	8	16	1-32	2	4
	Itraconazole	0.06-4	0.5	1	0.007-0.25	0.015	0.12
	Terbinafine	0.03-0.12	0.06	0.12	0.007- 0.06	0.015	0.03

Fluconazole showed a higher MIC value when compared to other drugs by both methods.

Terbinafine recorded the lowest MIC values.The MIC values were much lower when tested by microbroth dilution method. Terbinafine was found to be the most potent drug.

CONCLUSION

1. 170 clincally diagnosed cases of dematophytosis were subjected to mycological study

2. Male : Female sex ratio was 54%: 46%.

3. Maximum isolates of dermatophytes were from the age group of above20 years (70%) The post pubertal changes in harmones may be attributable to this finding.

4. Maximum number of Tinea capitis cases was found in the age group of >10 years. As universally reported Tinea capitis is a infection of children.

5. Culture positivity was 35.2%. Tinea unguium cases showed more positivity than other lesions(28%)

6. Trichophyton spp were the predominant isolates (93.9%) followed by Microsporum spp (5%) and Epidermophyton (3.3%).

7. Trichophyton rubrum was the most predominant isolate from skin(35.7%) and nail (28.5%).Adults males were found to be more susceptible to T.rubrum infection than children.

8. In Tinea corporis, Trichophyton rubrum was the most commonest isolate (46.6%).

9. In cases of Tinea cruris, Trichophton verrucosum and Epidermophyton floccosm were isolated in maximum numbers (33.3% each).

10. In Tinea capitis, Trichophyton violeceum was isolated maximally (44.4%)

11. In Tinea mannum , Trichophyon mentagrophytes was the main isolate (50%)

12. In Tinea pedis , Trichophyton tonsurans was the maximum isolated species(66.6%).

13. In Tinea barbae , 50% of isolates were Trichophyton mentagrophytes and 50% were Trichophyton verrucosum.

14. In tinea unguium , Trichophyton rubrum was the main isolate (28.5%) followed by Trichophyton mentagrophytes (23.8%) and Trichophyton verrucosum (19%).

15. Fluconazole showed a higher MIC value when compared to other drugs by both methods.

16. Terbinifine was found to be the most effective antifungal drug as evaluated by the agar dilution method and microbroth dilution method.

17. A marked reduction in the MIC range was noted when antifungal susceptibility testing was done by microbroth dilution method.

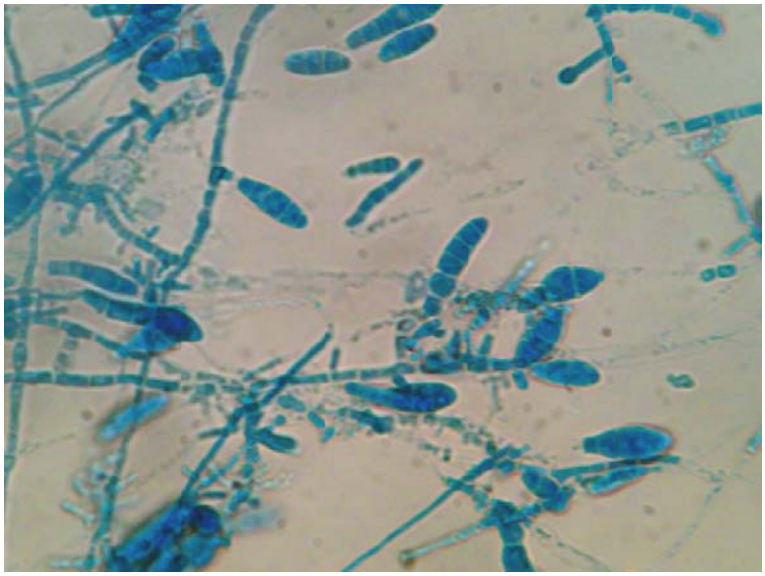
18. Terbinifine was the most effective of all the drugs tested.

In recent years several studies on In vitro susceptibility of dermatophytes to anti fungal drugs have been done and the results have shown considerable variation. This variability is probably due to important methodological differences among the laboratories.

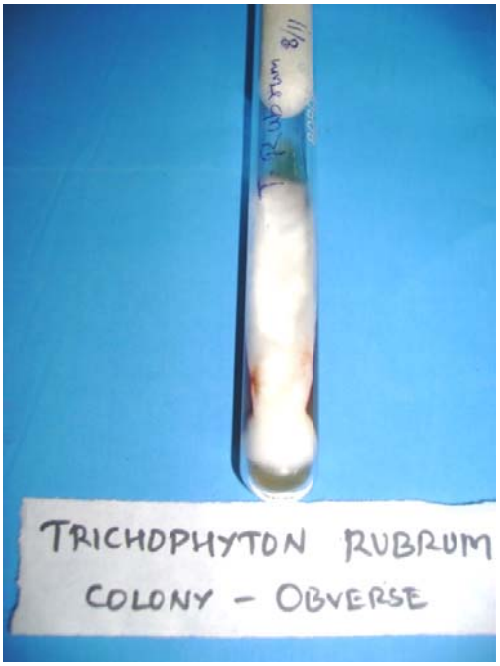
Our study demonstrated that several antifungal agents are very active against dermatophytes, although these results are species dependent. This can allow clinician to adopt different therapeutic options with high probability to successful results.

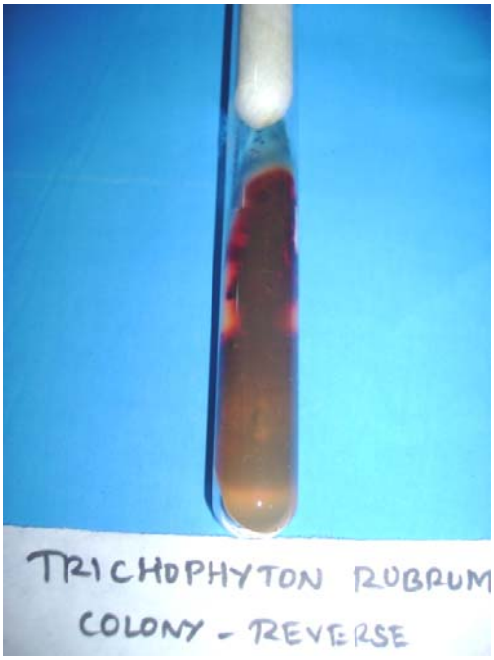
TINEA MANNUM

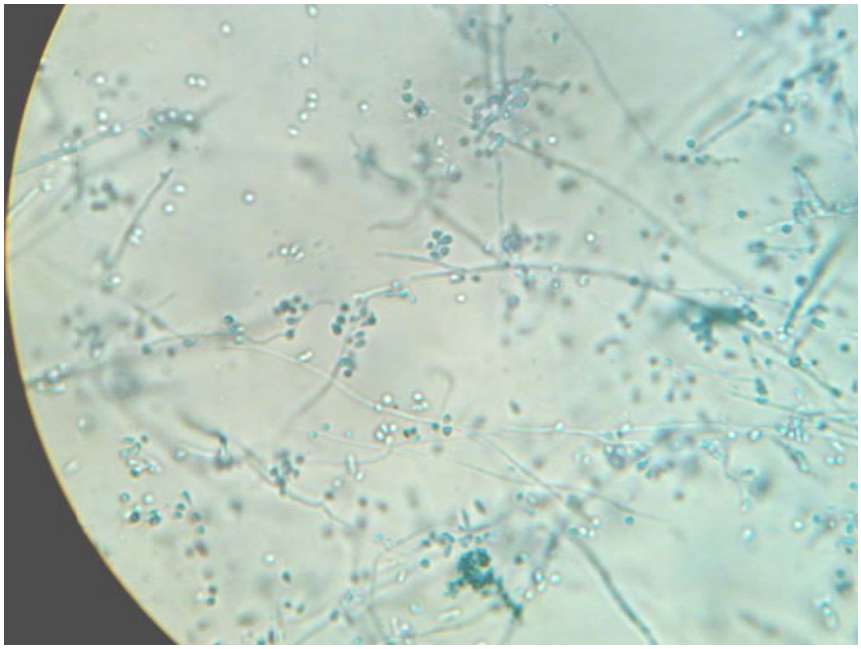
TINEA PEDIS



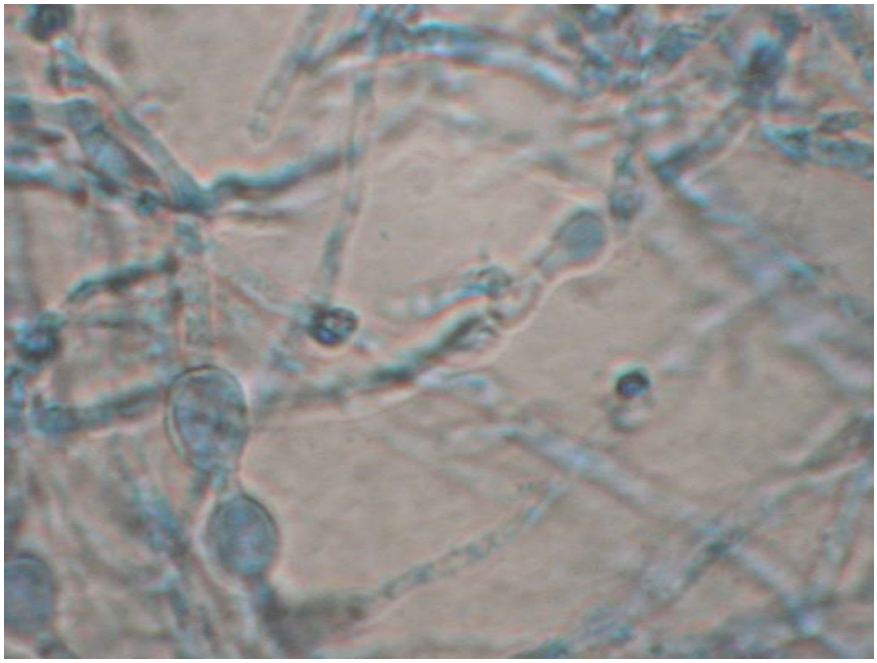
E.floccosum Microscopy





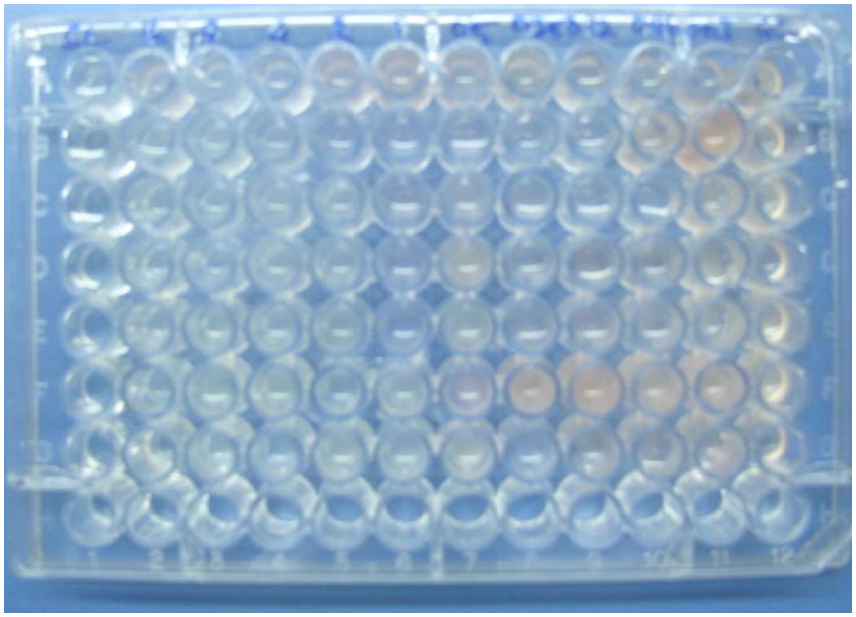


T.rubrum Microscopy



T.verrucosum Microscopy

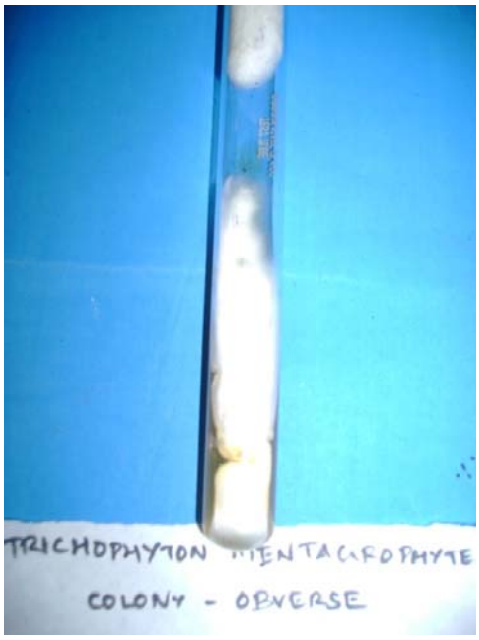
ANTI FUNGAL SUSCEPTIBILITY TESTING BY AGAR DILUTION METHOD

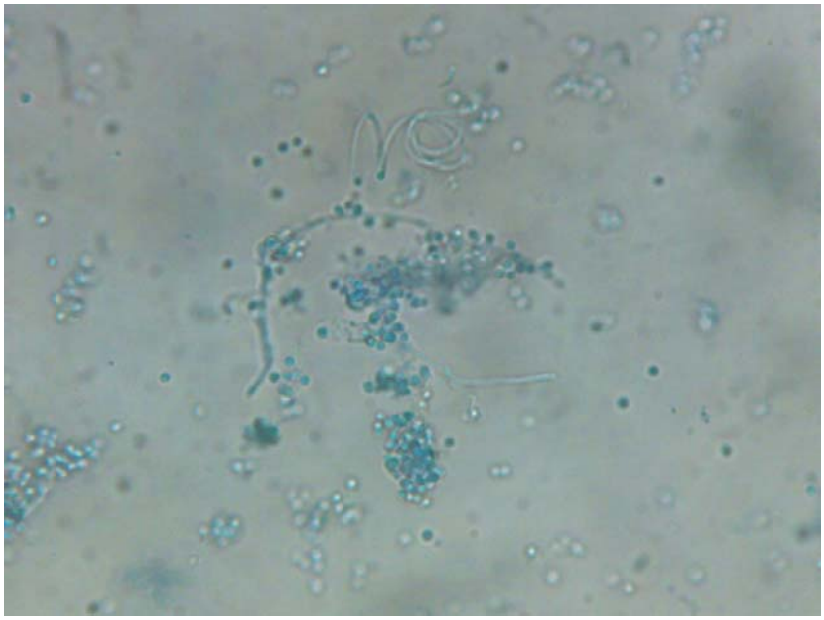


ANTI FUNGAL SUSCEPTIBILITY TESTING BY MICROBROTH DILUTION METHOD

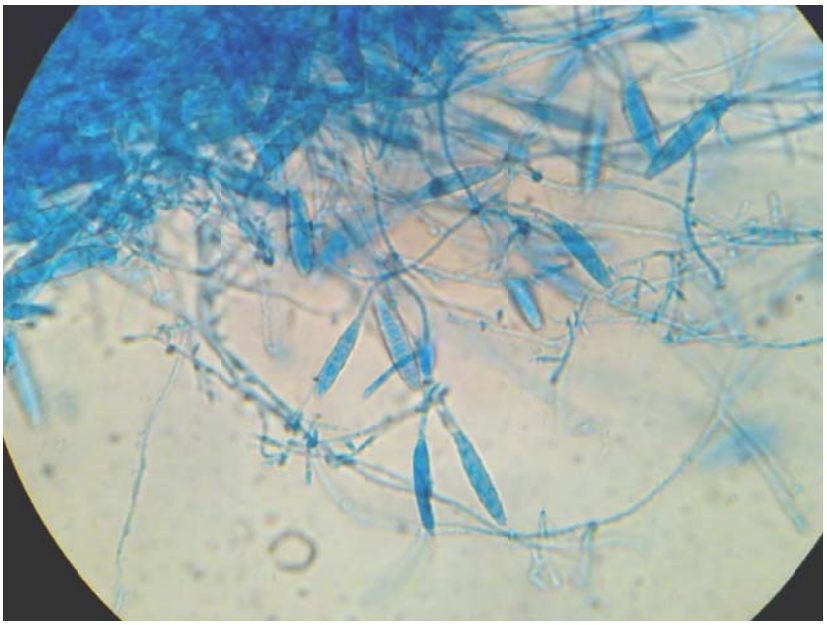
TINEA CORPORIS

TINEA UNGIUIM

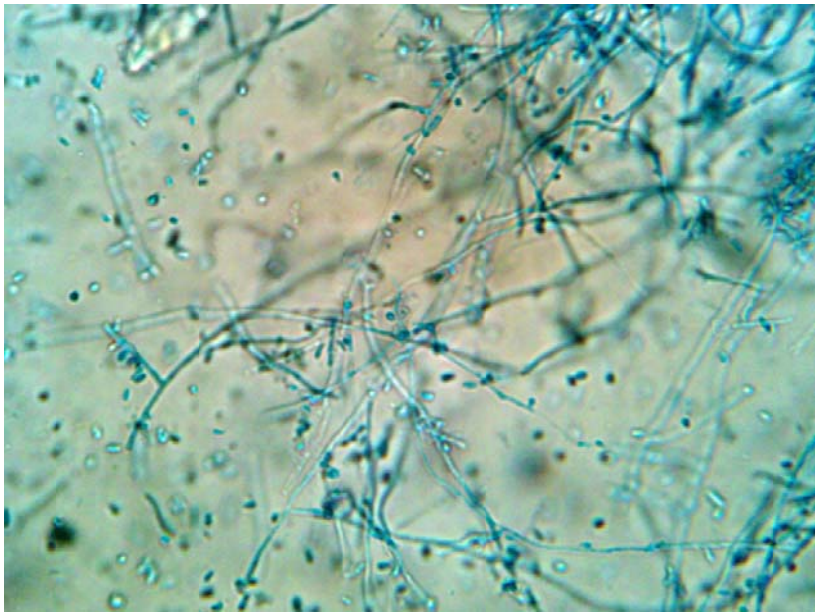




T.mentagrophytes Microscopy

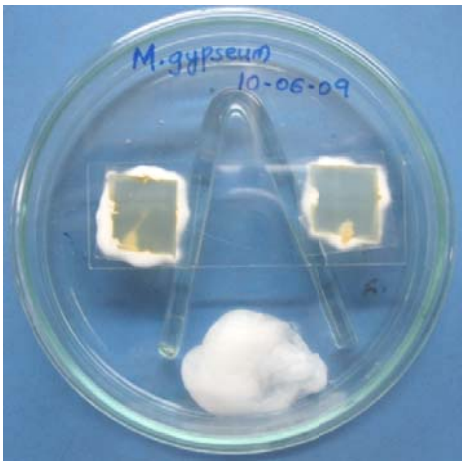


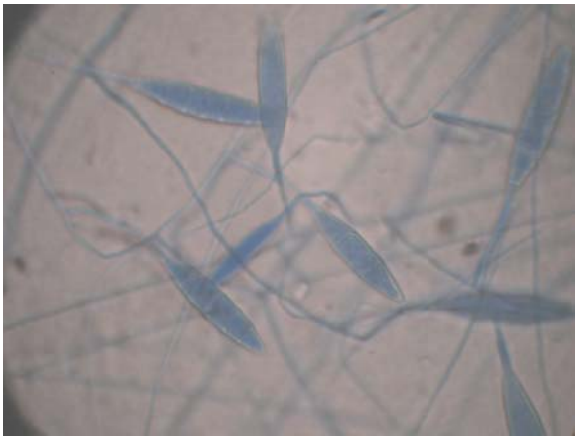
M.gypseum Microscopy



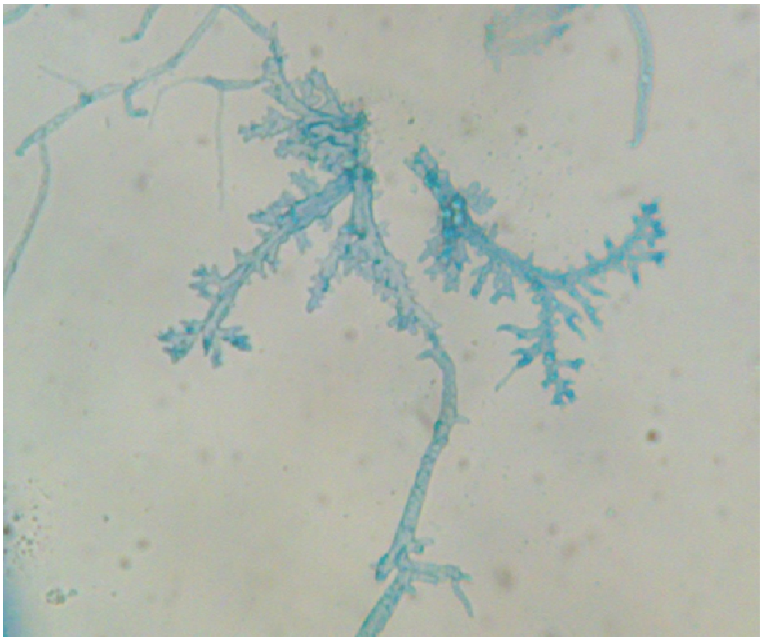
T.tonsurans Microscopy

SLIDE CULTURAL

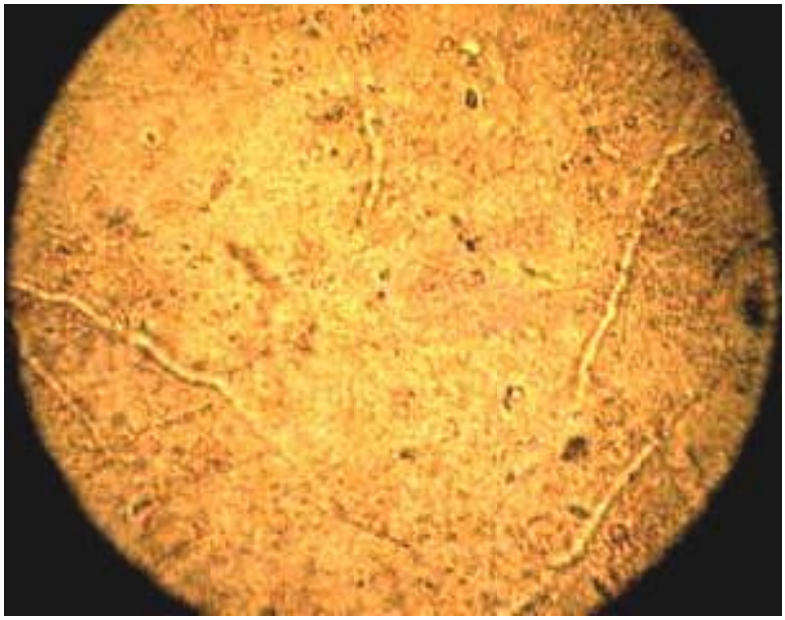




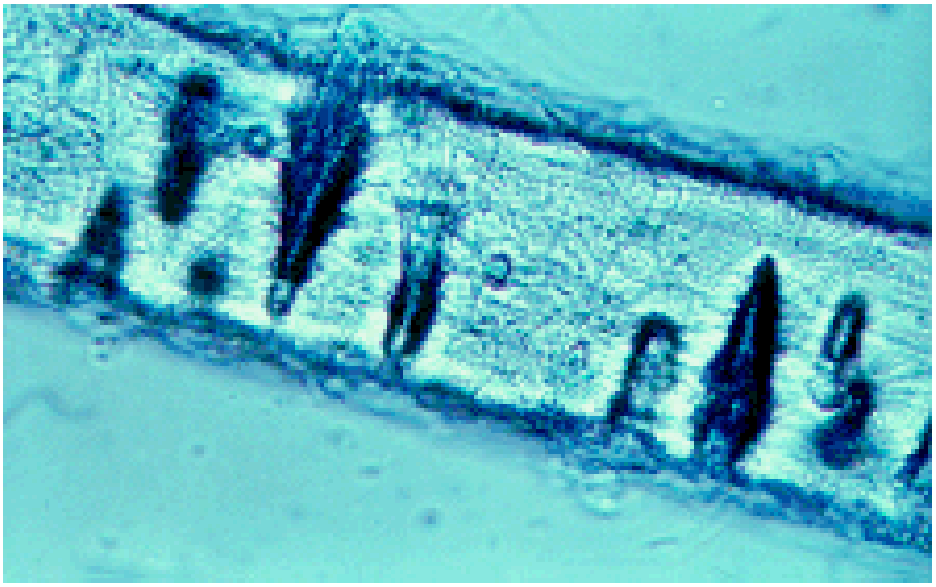
Microsporum Gypseum



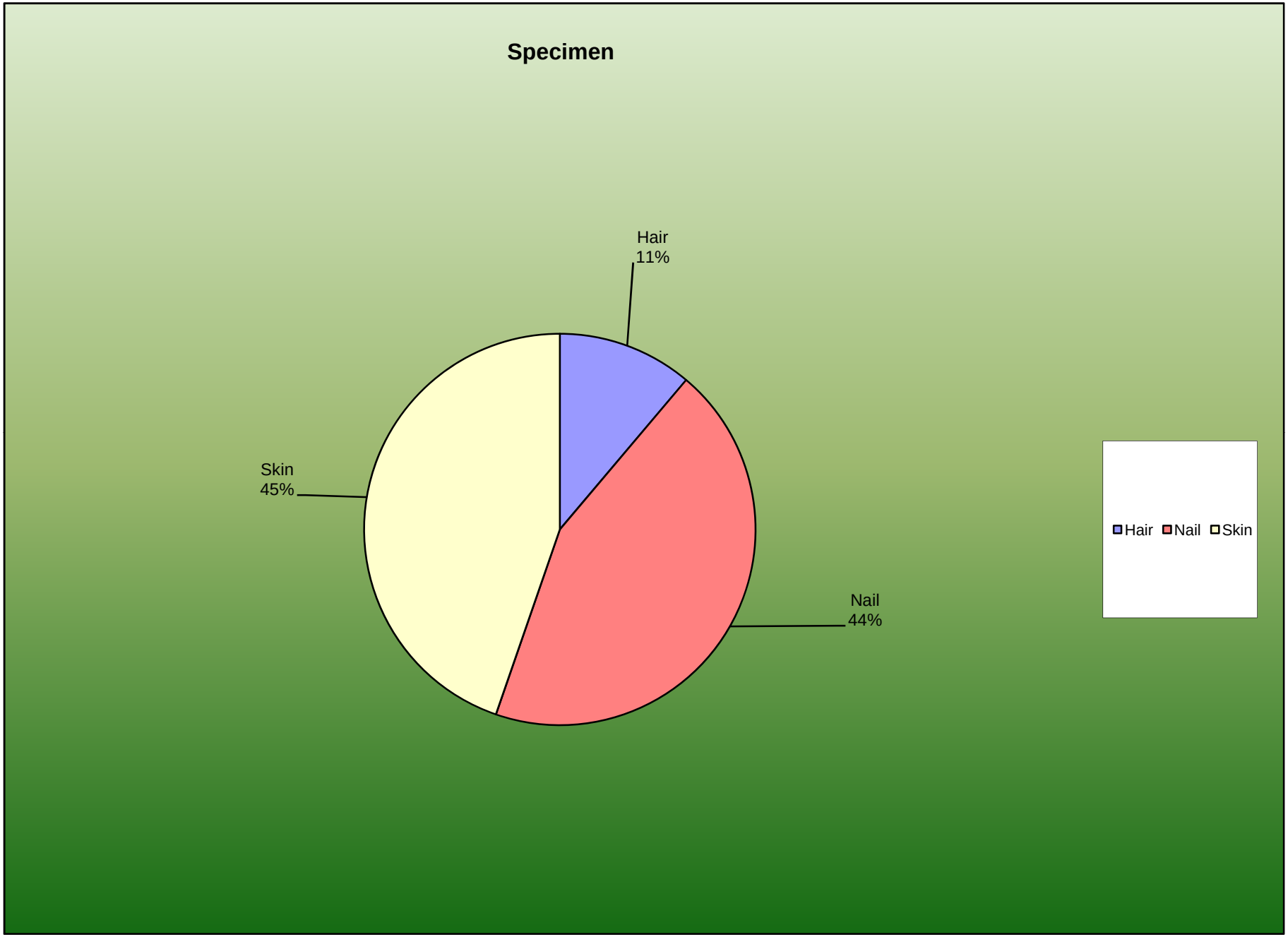
TRICHOPHYTON SHOENLEINII

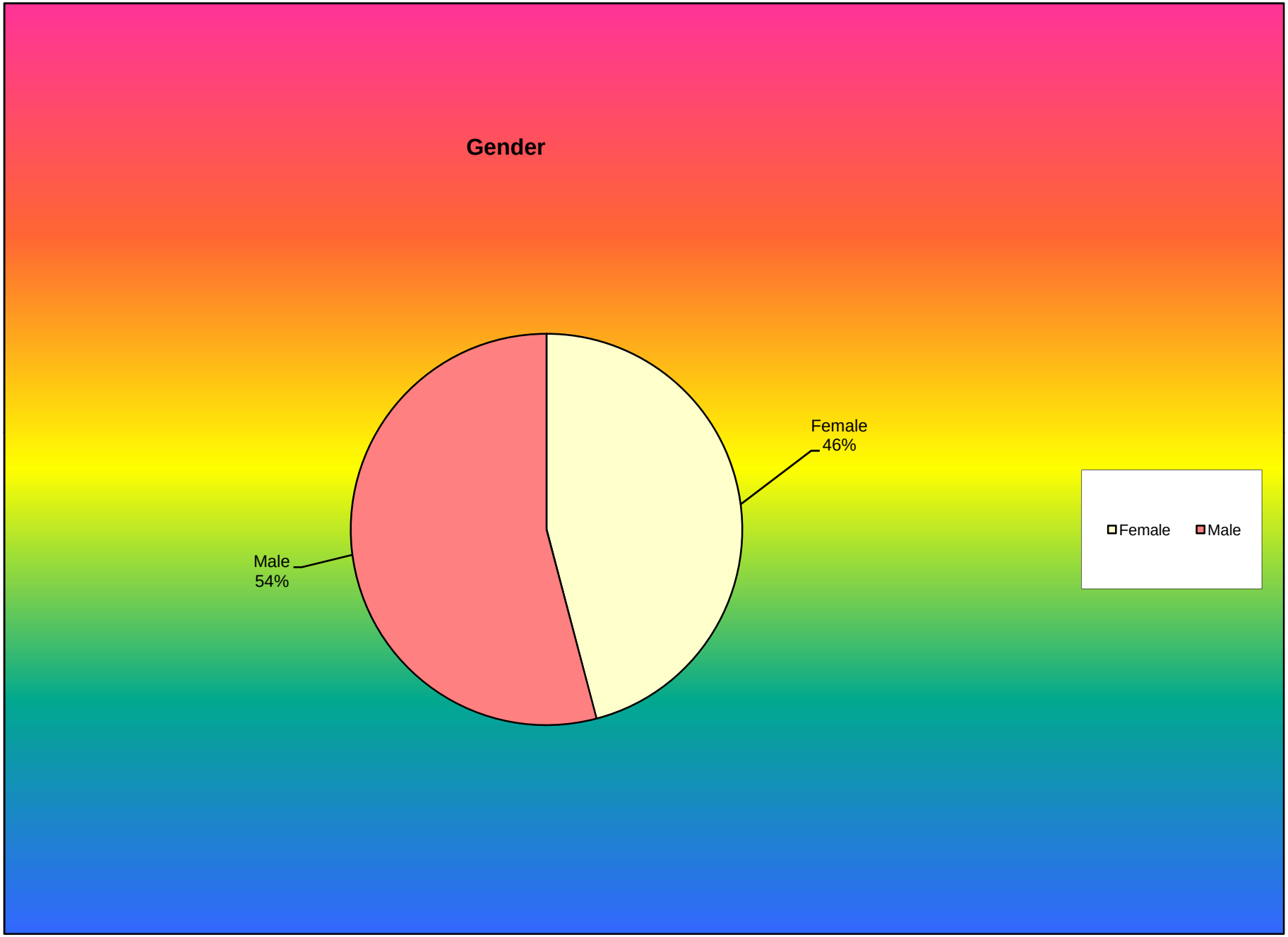


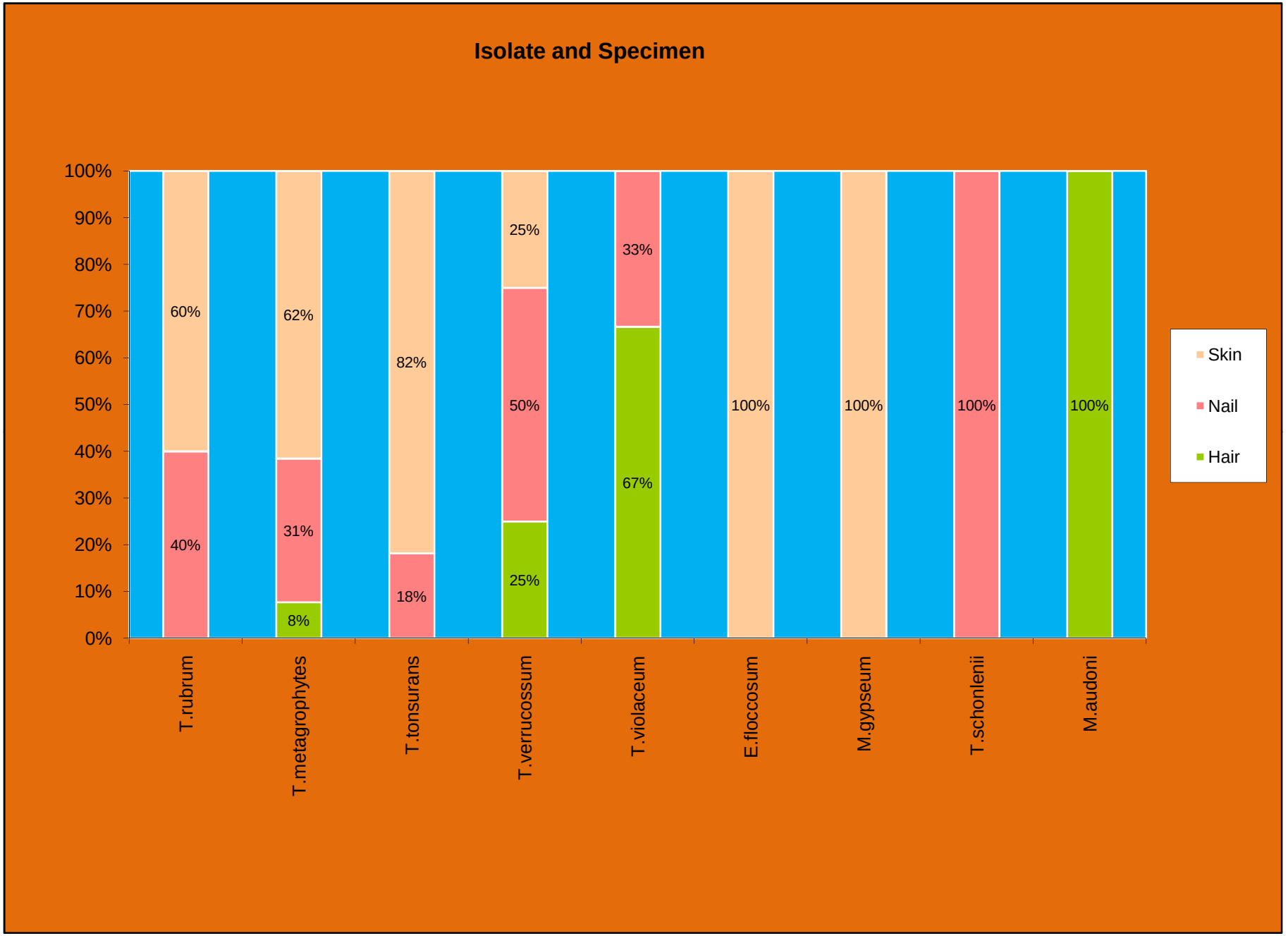
KOH MOUNT



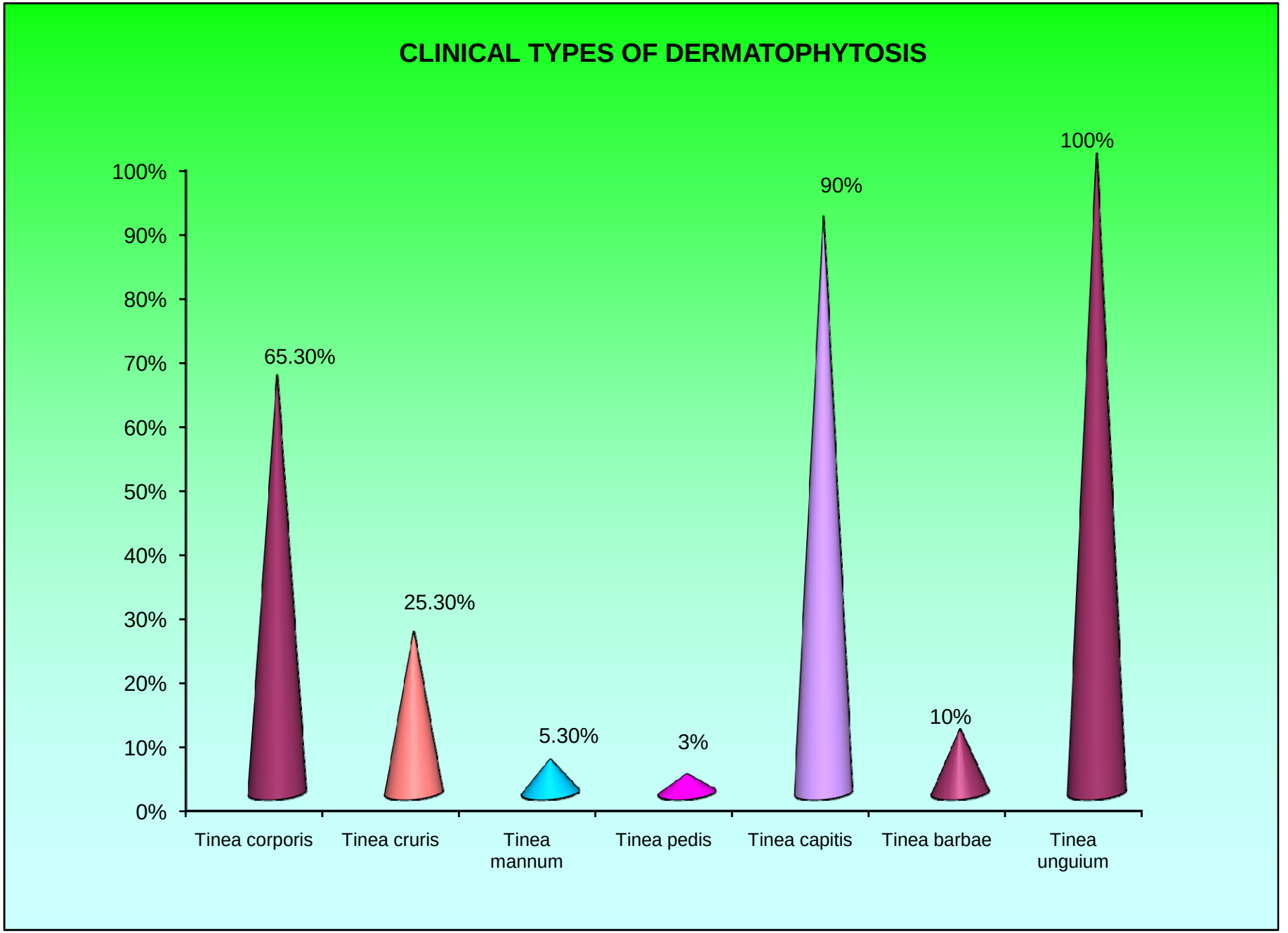
HAIR PERFORATION TEST


Specimen
Hair
11%
Nail
44%
Skin
45%
Hair
Nail
Skin


Gender
Female
46%
Male
54%
Female
Male


Isolate and Specimen
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
60%
40%
62%
31%
8%
82%
18%
25%
50%
25%
33%
67%
100%
100%
100%
100%
T.rubrum
T.metagrophytes
T.tonsurans
T.verrucossum
T.violaceum
E.floccosum
M.gypseum
T.schonlenii
M.audoni
Skin
Nail
Hair


Age Distribution
25%
20%
15%
10%
5%
0%
3%
19%
18%
17%
21%
16%
7%
<=10
11-20
21-30
31-40
41-50
51-60
>60


CLINICAL TYPES OF DERMATOPHYTOSIS
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
65.30%
25.30%
5.30%
3%
90%
10%
100%
Tinea corporis
Tinea cruris
Tinea mannum
Tinea pedis
Tinea capitis
Tinea barbae
Tinea unguium